

In the Literature

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This section of News and Views will present updates of recent advances in the medical and scientific literature.

Oocytes Matured *in vitro* May Improve Current Infertility Therapy

The polycystic ovarian syndrome (PCOS) is an incompletely understood disorder characterized by infertility due to anovulation, hyperandrogenism, and ovaries containing multiple immature cystic follicles. Conventional treatment for infertility due to PCOS has involved ovulation induction using agents such as clomiphene and gonadotropins. However, infertility due to PCOS is often refractory to these drugs. When a response is achieved, patients are at risk of developing ovarian hyperstimulation syndrome, which may result in ascites, oliguria, electrolyte disturbances, and thromboembolism. Chiang and colleagues at the Royal Victoria Hospital in Montreal have reported that modification of the technique of *in vitro* fertilization (IVF) with intracytoplasmic sperm injection (ICSI) can improve success rates in women with PCOS. The modification consists of inducing maturation of the oocyte *in vitro* prior to fertilization. The 20 women in their study were treated for 10 days with progesterone to produce a withdrawal bleed, followed 10-14 days later by a dose of human chorionic gonadotropin (hCG) to induce ovulation. After another 36h, eggs were harvested by aspiration from the surfaces of their ovaries. The oocytes were then allowed to mature *in vitro* in a medium enriched with gonadotropins and the patient's own serum. The investigators found that 84% of 249 oocytes (retrieved from the 20 women in a total of 25 treatments of IVF with ICSI) matured successfully *in vitro*. The pregnancy rate was 40% (10 pregnancies in 25 treatments). Moreover, three women whose infertility had previously been unsuccessfully treated with conventional IVF became pregnant using this new method. This evidence indicates that a bet-

ter infertility treatment may soon be widely available to women with PCOS, in whom conventional infertility therapies are often not successful.

(Chiang R-C, Gülekli B, Buckett WM, and Tan S-L. *NEJM*. (1999). 341: 1624-1626)

Mice Genetically Protected Against Oxidative Damage Live Longer

It has often been suspected that aging and senescence may in part result from the cumulative cellular effects of oxidative stress. In the mid-1990s, it was discovered that nematode worms, yeast, and fruit flies protected from oxidative stress by robust cellular antioxidant mechanisms have longer life spans. Now it has been shown, by Migliaccio and co-workers, that a specific mutation in mice produces a longer lifespan, also by conferring resistance to oxidative damage. This mutation is found in the gene encoding p66^{shc}, an adaptor protein that transduces signals from activated tyrosine kinase receptors to the nucleus. The researchers exposed mouse embryo fibroblasts (MEFs) to two forms of oxidative stress, namely ultraviolet (UV) light and hydrogen peroxide. They found that p66^{shc} is serine phosphorylated in MEFs in response to these oxidative stressors. They then compared wild type MEFs with MEFs in which one or both copies of the p66^{shc} gene were knocked out. Wild type MEFs underwent massive apoptosis in response to UV and peroxide. However, lower rates of apoptosis were seen among MEFs with one copy of p66^{shc} knocked out, and even lower rates were seen among MEFs with both copies knocked out. Interestingly, the capacity to proliferate was retained in the surviving knockout MEFs. In an attempt to clarify the role of p66^{shc} in the apoptotic response to oxida-

tive stress, the researchers investigated the interaction of the protein with p53 and p21. These proteins are known to be involved in cell cycle arrest and apoptosis in response to many insults, including oxidative damage. Further, it is known that the upregulation of p21 that occurs following exposure to peroxide or UV results from p53-dependent and -independent pathways. In this study, the researchers found that p53 was upregulated to a comparable degree in both wild type and p66^{shc} knockout MEFs in response to oxidative stress. However, in p66^{shc} knockout MEFs, the p21 response was partially attenuated when compared with the p21 response in wild type MEFs exposed to the same degree and type of oxidative stress. This finding suggests that the p53-independent pathway of p21 activation is disrupted by the loss of p66^{shc}. Further investigation revealed that knockout of p21 alone did not affect the response of the MEFs to oxidative stress, suggesting that the protective effects of p66^{shc} knockout could not solely be attributed to disruption of p21 activation. As predicted by previous studies, knockout of p53 produced MEFs that were resistant to apoptosis. Taken together, these findings led the researchers to speculate that oxidative stress can activate one of two pathways: one in which p53 stimulates apoptosis and another in which p21 protects from apoptosis, through p53-dependent and -independent activation. Finally, the investigators treated wild type mice and mice with both copies of p66^{shc} knocked out with paraquat, a drug that generates intracellular superoxide anions. While 5 of 5 wild type mice died in 48 hours, only 2 of 5 knockout mice died in the same period, and one survived for several weeks. Cumulative statistical analysis showed that this difference translated into a 40% increase in lifespan in the knockout mice. These findings have at least two important implications. First, they extend the findings of previous studies which implicated a role for oxidative damage in the aging of mammals. Second, they appear to contradict the traditional evolutionary dogma that aging is a post-reproductive process: genes acting before a reproductive age to confer a shortened lifespan (p66^{shc} acts both before and after reproduction) should be under great selective pressure to be eliminated from the population.

(Migliaccio E, Giorgio M, Mele S, et al. *Nature*. (1999). 402: 309-313)

NSAIDs Inhibit Angiogenesis *in vitro*

Angiogenesis (the formation of new blood vessels from existing ones) is a fundamental process that plays a key role in wound healing. There is also mounting evidence that it is critical in the development of cancer, by providing growing primary tumours and metastases with a source of oxygen and nutrition. Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin are known to be chemopreventative against colon cancer for unknown reasons. Jones and colleagues hypothesized that this effect may result from NSAID-mediated inhibition of angiogenesis. They

studied the ability of NSAIDs to inhibit angiogenesis *in vitro* using rat and human endothelial cells. Both vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) stimulate angiogenesis in these cells. This effect was blocked by the addition of indomethacin, a "traditional NSAID" that inhibits both COX-1 and COX-2 isoforms of cyclooxygenase. It was also abolished by NS-398, a new COX-2-selective NSAID. It is known from previous work that the mitogen-activated protein kinase (MAP kinase) ERK2 must undergo nuclear translocation in endothelial cells for angiogenesis to occur. Both indomethacin and NS-398 inhibited the kinase activity of ERK2 (*in vitro* in a cell-free preparation); similarly, both drugs inhibited the nuclear translocation of ERK2 (as detected by immunofluorescence microscopy) in endothelial cells exposed to these drugs. Since many of the effects of NSAIDs are attributable to COX inhibition, the researchers added prostaglandin E₂ (PGE₂) and prostacyclin (PGI₂) to endothelial cell cultures. These compounds partially reversed the inhibition of angiogenesis induced by NS-398 but, intriguingly, not by indomethacin. Taken together, these findings indicate that endothelial cell prostaglandins are important for angiogenesis, but that prostaglandin-independent angiogenesis can occur and is also inhibited by non-selective NSAIDs via a pathway not involving COX-1 or COX-2. This study has at least two important ramifications. First, COX-2-selective inhibitors, which are touted as less likely to produce gastric and duodenal ulcers, may in fact inhibit the angiogenesis involved in ulcer healing. Second, the chemoprotective effect of NSAIDs against colon cancer may now, at least in part, be explained. NSAIDs may find use in the future in the prevention and/or treatment of other cancers.

(Jones MK, Wang H, Peskar BM, et al. *Nature Med*. (1999). 5: 1418-1423)

Importance of C-reactive Protein and Complement in Acute Myocardial Infarction

It is known that both complement components and C-reactive protein (CRP), an acute phase protein, are deposited in infarcted myocardial tissue, from both clinical and laboratory studies. Serum CRP peaks rapidly in acute illnesses associated with tissue damage and inflammation, including acute myocardial infarction (AMI). It has been shown that the peak serum CRP concentration following AMI correlates with patient outcome, with higher CRP concentrations conferring a poor prognosis. Furthermore, human CRP, but not rat CRP, is a potent activator of the classical complement pathway, a cascade of proteolytic reactions that culminates in cell membrane damage and inflammatory cell recruitment. Consequently, Griselli and co-workers hypothesized that CRP-mediated activation of the classical complement pathway may underlie a portion of the myocardial damage resulting from AMI. Experimental AMI was induced in Wistar rats by ligation of the left anterior descending (LAD)

coronary artery. Infarctions were confirmed electrocardiographically. In the five days following the infarction, the rats were injected repeatedly with either human CRP, cobra venom factor (a protein that depletes serum complement), both, or the buffer solution. After this period, infarct sizes were measured by sectioning the myocardial tissue and treating it with nitroblue tetrazolium (NBT), which stains only viable tissue blue. Rats injected with human CRP were less well and had reproducibly larger (by 40%) infarcts than rats injected with buffer solution. In contrast, rats injected with cobra venom factor up to 24 hours prior to coronary artery ligation had infarcts that were 60% smaller than rats injected with buffer solution. Addition of human CRP to the latter experiment did not abrogate the reduction in infarct size; this indicates that the damaging effect of human CRP is dependent on the presence of complement in this model. A further experiment was conducted in which human serum amyloid protein (SAP), an acute phase protein very similar in structure to human CRP, was injected. This did not have any of the adverse effects associated with the injection of human CRP, indicating that these effects were specific to human CRP. Most interestingly, from a clinical perspective, was the finding that the addition of cobra venom factor as much as two hours after LAD ligation reduced infarct size by almost 50%. On histologic analysis, the researchers found that human CRP, rat CRP, and complement were deposited in the infarcted myocardium, and that complement components were only present if human CRP was also present. Together, these findings suggest that complement and CRP play an important role in the development of myocardial damage following AMI, and that it is possible to interfere with this process by depleting serum complement even after the onset of ischemia. Hence, they point toward a possible future therapeutic intervention for AMI.

(Griselli M, Herbert J, Hutchinson WL, et al. *J Exp Med.* (1999). 190: 1733-1739)

The DNA Sequence of Human Chromosome 22: The First Sequencing Landmark of the Human Genome Project

The human genome is neatly packaged into 23 pairs of chromosomes, with 22 pairs of autosomes and 1 pair of sex chromosomes. Chromosome 22 is one of the smallest human autosomes and it is comprised of 1.6 – 1.8% of the genomic DNA. It is one of the five acrocentric chromosomes. The short arm of chromosome 22 consists of the tandemly repeated ribosomal RNA genes as well as other tandem repeat arrays; however, there is no evidence of any protein encoding genes in that region. In contrast, its long arm is rich in genes compared with other chromosomes. To date, there are thought to be over twenty human disorders associated with changes to genes on this chromosome. Gene dosage alteration of chromosome 22 has been implicated in a number of human diseases, including cat eye syn-

drome, velocardiofacial/DiGeorge syndrome, schizophrenia, and spinocerebellar ataxia 10. The sequence that was reported in a recent issue of *Nature* consists of 33.4 million bases containing at least 545 genes. However, the sequences of eleven small gaps were unable to be determined with existing cloning techniques. Nevertheless, this effort generated the largest contiguous segment of DNA sequence known to date and it represents the first sequencing landmark of the human genome project. In practical terms, this DNA sequence will provide a framework within which we will be able to map differences between individuals that make us unique, as well as changes that represent disease states. The next challenge following the completion of the human genome project (projected in the year 2002) is to translate this information into tangible applications.

(Dunham I, Shimizu N, Roe BA, Chissov S, et al. *Nature.* (1999). 402: 489-495)

The Identification of a Promising Antibiotic Candidate

The increasing resistance of pathogenic microbes to antibiotics raises the possibility of an alarming scenario in which doctors are powerless to treat many bacterial infections. Even vancomycin, a longtime antibiotic of last resort, seems to be losing its effect: vancomycin resistance has emerged in enterococci. Researchers have discovered a promising replacement antibiotic that has been used as a food preservative for over fifty years. The compound, nisin, is a peptide of the lantibiotic family (lanthionine-containing antibiotics) and it is produced by certain strains of *Lactococcus lactis*, the bacteria that can turn milk sour. Nisin is known to be non-toxic to humans yet it possesses high bactericidal activity. Its mechanism of action was thought to be permeabilization of the bacterial plasma membrane through the formation of pores. In a recent issue of *Science*, a Dutch-German team of scientists demonstrated that nisin binds the membrane-anchored cell wall precursor Lipid II, the same target as vancomycin. First, the researchers found that nisin resembles magainin, a peptide antibiotic derived from frogs, in that it kills *Micrococcus flavus* bacteria by forming pores in the cell membrane. Secondly, further experiments showed that vancomycin inhibited nisin's membrane leakage activity against intact bacterial cells but it had no effect on magainin. These results suggested that vancomycin and nisin are competing for the same target, namely Lipid II. In a series of tested systems, the interaction between nisin and Lipid II was demonstrated to be specific; however the precise mechanism of pore formation through Lipid II remains to be elucidated. The low effective dose of nisin makes it desirable compared to other known peptide antibiotics. In addition, no nisin-resistant bacterium has been identified to date. This discovery holds a promise that nisin may one day replace vancomycin as a broad-range antibiotic.

(Breukink E, Wiedemann I, van Kraaij C, et al. *Science.* (1999). 286: 2361-2364)

Primate Cloning by Embryo Splitting

Animal cloning was merely a concept in science fiction until the birth of the sheep "Dolly" in 1997. This remarkable achievement was followed by the successful cloning of cattle, mice, and goats. Cloning by nuclear transfer using blastomere nuclei in macaques has also been described; however, the nuclear transfer technique results in genetic chimeras with mitochondrial heterogeneity. In a recent issue of *Science*, Chan and colleagues reported the birth of the first non-human primate clone produced by embryo splitting. One hundred and seven rhesus embryos were split to create 368 multiples. Specifically, an eight-cell embryo was split to produce a set of identical quadruplet embryos each consisting of two blastomeres. A pair of the quadruplet embryos was then transferred into two fertile surrogates; both became pregnant. One gestated a pregnancy with a placental sac devoid of fetal tissue while the other pregnancy was uneventful and culminated in the birth of Tetra, the first rhesus monkey cloned by embryo splitting, at 157 days. Microsatellite-based polymerase chain reaction demonstrated that the "blighted" pregnancy and Tetra were genetically identical. A total of thirteen embryo transfers were performed, which resulted in four pregnancies (31% success rate), 1 fetal sac, and one live birth (8%). In contrast, in vitro fertilization controls produced eight pregnancies (53%), 10 fetal sacs, and 6 live births (40%). Apoptosis was found to be higher in split embryos (especially in the inner cell mass) than in controls, likely contributing to the higher miscarriage rate. Quadruplets appear to be the upper viability limit for offspring survival. Furthermore, larger sets of identical embryos showed decreased developmental potential. Cloning by embryo splitting produces genetically identical offspring, which are valuable tools in the study of disease and innovative therapies.

(Chan AWS, Dominko T, Luetjens CM, et al. *Science*. (2000). 287: 317-319)

Correlation Between the Risk of Spontaneous Abortion and Caffeine Consumption

The consumption of caffeine during pregnancy and its possible relationship with spontaneous abortion is a controversial health issue. Some studies have indicated that moderate to heavy caffeine intake is associated with a risk of fetal loss while others have found no evidence of increased risk. Previous studies used questionnaires to ascertain caffeine consumption, which may not be an accurate assessment. In a nested-case control study, Klebanoff and colleagues used a biological marker, serum paraxanthine (the primary caffeine metabolite) to measure the amount of caffeine present in the body. The serum paraxanthine levels of 591 women who had spontaneous abortions at less than 140 days gestation and 2558 matched women from the same clinic who gave birth to live infants at 28 weeks gestation or later, were measured and compared. Results demonstrated that the mean concentration of serum paraxanthine was

significantly higher in the women who had spontaneous abortions than that in the controls (752 vs. 583 ng/mL, $p < 0.001$). However, the risk of spontaneous abortion is not increased until extremely high serum paraxanthine levels (1845 ng/mL) are reached. Since there is no precise way to equate serum paraxanthine level with the amount of caffeine consumed, it is not yet possible to determine how much caffeine is safe during pregnancy. A previous study by the same authors found that the highest caffeine intake was 1530 mg (approximately 15 cups of coffee) and the highest measured serum paraxanthine concentration was found to be 1165 ng/ml (substantially lower than the 1845 ng/ml value in this study). These high levels of caffeine intake may be reflective of the lifestyle in the 1960s (when the samples were obtained), and the consumption of such high levels of caffeine is probably not a common practice today. In summary, the results of this study suggest that the risk of spontaneous abortion is associated with extremely high caffeine consumption and that moderate consumption is unlikely to constitute a significant risk.

(Klebanoff MA, Levine RJ, DerSimonian R, et al. *NEJM*. (1999). 341: 1639-1644)

The Hope Trial: Effects of an ACE Inhibitor on Death from Cardiovascular Causes, Myocardial Infarction, and Stroke in High Risk Patients

(reviewed by Marshall Hay, B.Sc. OT1)

Recently, New England Journal of Medicine's web site (www.nejm.org) published a paper studying the effects of ACE inhibitors in high-risk patients. The online publication preceded its journal appearance (expected to appear January 20, 2000) because of the potential therapeutic implications. As described elsewhere, the Heart Outcomes Prevention Evaluation (HOPE) Study Investigators examined 9297 high risk patients (55 years of age or older) who had vascular disease or diabetes plus at least one other cardiovascular risk factor (hypertension, elevated total cholesterol levels, low HDL cholesterol levels, smoking, or microalbuminuria) who were not known to have a low ejection fraction or heart failure. Subjects were randomly assigned to receive ramipril (100 mg po OD) or placebo for a mean of five years. Results demonstrated an overall reduction of 22 percent in the primary outcome of myocardial infarction, stroke, or death from cardiovascular causes. Additionally, those receiving ramipril had higher rates of coronary revascularization, lower rates of cardiac arrest and heart failure, and lower rates of complications related to diabetes and of diabetes itself. These results may significantly broaden the spectrum of patients who are likely to benefit from ACE inhibitors.

(The HOPE Study Investigators. *NEJM*. (2000). 342: 145-153)