



*Logo: Life's blood flows through the hourglass; the stopcock represents the alteration of aging and disease as biomedical research progresses.*

## Report of the Director

Dear Friends,

OFAS was founded in 1961, making this our 43<sup>rd</sup> year in biomedical research. We are pleased to report that the Foundation is contributing as much as ever to scientific knowledge.

We are especially delighted that we continue to make excellent use of the Serum Treasury and the coordinated Kaiser Permanente Medical Care Program database, which has been called by the World Health Organization "one of the most valuable resources currently available in biological banking." As you will read inside, the Serum Treasury continues to make possible diverse contributions to knowledge of the role of infectious agents in cancer and heart disease. Research with the Serum Treasury also resulted recently in three publications on the role of Insulin-like Growth Factor-1 and Tumor Necrosis Factor in relation to breast cancer risk. We are grateful to have distinguished scientists with whom to collaborate on this important work.

Inspired by the established value of the human Serum Treasury, OFAS has initiated the Pet Animal Serum Treasury (PAST) in cooperation with the Animal Cancer Foundation. We anticipate that PAST will become an equally valuable resource for research into the causes of cancer and other diseases in pets.

OFAS continues its seminal research on how restricting (but not eliminating) the essential amino acid methionine extends the lifespan of various strains of rats; it does this without making them irritable, which is what normally happens with severe calorie restriction, the time-tested, gold-standard of lifespan extension. Additional investigation is underway into the mechanism(s) by which methionine restriction extends lifespan. Its ability to increase levels of the potent antioxidant glutathione seems to be a promising answer.

In the area of research into the treatment of human hair loss, OFAS has made an unexpected observation. Our research has determined that the miniaturized (vellus) hairs from balding human scalp regain the ability to grow robustly when transplanted to immunodeficient mice. Nothing like a change of environment to show that an effete, 'peach fuzz' hair follicle is ready to perform again!

If you are on our mailing list, you already know about our VitaLongevity™ Newsletter, and we hope you find it useful in making your life as long and healthy as possible. The next issue of this quarterly publication will be in your hands soon.

With sincere appreciation for your support in the diverse forms it takes,

Norman Orentreich, MD, FACP  
Director

David S. Orentreich, MD  
Associate Director

## Published Results: 2002-2004

**ST** Denotes studies using the Serum Treasury.

### Infectious Agents, Cancer, and Heart Disease

#### **ST** The Importance of *Helicobacter pylori* Strain in Stomach Cancer

Stomach cancer is the second most prevalent cancer in the world, especially in Asia, Latin America, and some European countries. This cancer is strikingly associated with previous infection by the bacterium *H. pylori*, one particular strain (CagA+) of which is more strongly associated with risk. Our study showed that tumors from persons previously infected with this virulent strain were more apt to show a mutation of the p53 tumor suppressor gene, a well-studied gene that is frequently mutated in various types of human cancer. Shibata A, Parsonnet J, Longacre TA, Garcia MI, Puligandla B, Davis RE, Vogelmann JH, Orentreich N, Habel LA.

CagA status of *Helicobacter pylori* infection and p53 gene mutations in gastric adenocarcinoma. *Carcinogenesis* 23(3):419-424, 2002.

Collaborating Institutions: Stanford University School of Medicine, Kaiser Permanente Medical Care Program, and National Cancer Institute

#### **ST** The Complex Role of Infection and Inflammation in Myocardial Infarction Risk

Because heart disease is not always accounted for by traditional risk factors (e.g., lipids, smoking, hypertension, etc.), it is important to discover other contributory factors, such as treatable infections. To this end we investigated the role of infectious agents in persons who had experienced a myocardial infarction (MI) and who had serum stored in the Serum Treasury. C-reactive protein (a marker of inflammation related to infection) was significantly associated with MI but only in those over age 51. No connection between either *Chlamydia pneumoniae* or cytomegalovirus and MI was observed; further, there was no association between these infections and the level of C-reactive protein. Witherell HL, Smith KL, Friedman GD, Ley C, Thom DH, Orentreich N, Vogelmann JH, Parsonnet J. C-reactive protein, *Helicobacter pylori*, *Chlamydia pneumoniae*, cytomegalovirus and risk for myocardial infarction.

*Annals of Epidemiology* 13(3):170-177, 2003.

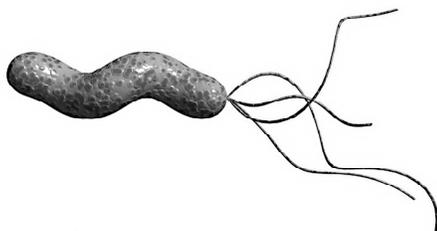
Collaborating Institutions: Stanford University School of Medicine and Kaiser Permanente Medical Care Program

#### **ST** *Helicobacter pylori* Infection Reduces the Risk of Subsequent Adenocarcinoma of the Esophagus

Infection with *H. pylori* carries at least one benefit: a substantially lowered risk of esophageal cancer. Using frozen samples from the Serum Treasury, we looked for antibodies to *H. pylori* as proof of previous infection in 52 patients who developed adenocarcinoma of the esophagus. We also checked for antibodies in 551 controls who had other diseases, i.e., lymphoma, colon cancer, gastric cancer, or heart disease. Only 38% of the persons who later developed cancer of the esophagus had antibodies whereas 62% was the average rate of infection for the control samples. We also found that infection with the particularly virulent Cag+ strain of *H. pylori* did not modify the protective effect. That cigarette smoking was a strong independent risk factor for esophageal cancer was no surprise..

de Martel C, Llosa AE, Farr SM, Friedman GD, Vogelmann JH, Orentreich N, Corley DA, Parsonnet J. *H. pylori* infection and risk of subsequent adenocarcinoma of the esophagus. *Journal of Infectious Diseases*, in press

Collaborating Institutions: Stanford University School of Medicine and Kaiser Permanente Medical Care Program



One of some two dozen species of *Helicobacter* that reside in the intestinal tracts of animals and human beings, *H. pylori* is between 2.5 and 5.0 microns long and lives beneath the mucus layer of the stomach.

Courtesy of Luke Marshall, Helicobacter Foundation

**ST Infection with *Chlamydia pneumoniae* Does Not Cause, But May Influence the Development of, Multiple Sclerosis**

Some people with Multiple Sclerosis (MS) have antibodies to the bacterium *Chlamydia pneumoniae*; others do not. To determine if previous infection with *C. pneumoniae* increases the risk of developing MS, our study looked for markers of infection in frozen serum of persons who developed MS up to 20 years after donating serum, either to the OFAS Serum Treasury or to the Department of Defense Serum Repository. The likelihood of developing MS after this infection was not increased, but the results allow for the possibility that infection somehow modifies the risk.

Munger KL, DeLorenze GN, Levin LI, Rubertone MV, Vogelman JH, Peck CA, Peeling RW, Orentreich N, Ascherio A.

A prospective study of *Chlamydia pneumoniae* infection and risk of MS in two US cohorts. *Neurology* 62:1799-1803, 2004.

Collaborating Institutions: Harvard School of Public Health, Kaiser Foundation Research Institute, Walter Reed Army Institute, US Army Center for Health Promotion and Preventive Medicine, US Army Physical Disability Agency, National Laboratory for Sexually Transmitted Diseases, National Microbiology Laboratory, and Harvard Medical School

*Infection and Multiple Sclerosis*

**ST Menopausal Status Affects Breast Cancer Risk Relative to Insulin-like Growth Factor-1**

IGFs (Insulin-like Growth Factors) and their binding proteins (IGFBPs 1, 2, and 3) play a role in the induction and progression of various cancers (*e.g.*, colon, prostate). Because estrogen both regulates and is influenced by the IGF family, we studied the relationship of breast cancer risk relative to IGF and its binding proteins in pre- and postmenopausal women. Risk was increased in premenopausal women who had elevated levels of IGF-1 and IGFBP-3. On the positive side, elevated levels of IGFBP-2 correlated with decreased risk of postmenopausal breast cancer.

Krajcik RA, Borofsky ND, Massardo S, Orentreich N.

Insulin-like growth factor-1 (IGF-1), IGF-binding proteins, and breast cancer. *Cancer Epidemiology, Biomarkers and Prevention* 11(12):1566-73, 2002.

*Breast Cancer and Insulin-like Growth Factor*

**ST Breast Cancer Risk is Not Increased by TNF Receptor Levels**

Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) is critical in maintaining resistance to infection and malignancy, and its beneficial action can be blocked by soluble TNF receptors. Using the OFAS Serum Treasury, we found that TNF receptor levels rise with age and with body mass index (BMI), but that neither abnormally high nor high-normal levels are associated with increased risk of breast cancer.

Krajcik RA, Massardo S, Orentreich N.

No association between serum levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) or the soluble receptors sTNFR1 and sTNFR2 and breast cancer risk. *Cancer Epidemiology, Biomarkers and Prevention* 12(9):945-46, 2003.

**Balding Hairs Grow Long and Thick on Immunodeficient Mice**

Because immunodeficient mice do not reject foreign tissues, they will accept transplants of human hairs that can then be studied. We transplanted both miniaturized and normal hair follicles from scalp affected by common balding. Our study found that miniaturized hair follicles can quickly regenerate once removed from the human scalp; in fact they grew as well as or better than the transplanted normal, non-balding hair follicles as assessed by their diameter and length achieved at 22 weeks.

Krajcik RA, Vogelman JH, Malloy VL, Orentreich N.

Transplants from balding and hairy androgenetic alopecia scalp regrow hair comparably well on immunodeficient mice.

*Journal of the American Academy of Dermatology* 48(5):752-59, 2003.

*Hair Regrowth*

## Insulin-like Growth Factor

### **ST** Disease Risk Can be Assessed by a Single Measurement of Insulin-like Growth Factor-1 in Stored Serum

Several common cancers (prostate, breast, colorectal, and lung) are associated with high serum levels of Insulin-like Growth Factor-1 (IGF-1), whereas low levels of this growth factor are associated with osteoporosis, impaired cognition, and heart disease. In anticipation of studies of these diseases, we evaluated the reliability of assays of IGF-1 in frozen serum samples from the Serum Treasury. Our study used samples from the same persons both fresh samples and stored up to 16 years. We found that each participant's serum level of IGF-1 remained relatively constant and, confirming previous results, that IGF-1 levels were lower in persons over 45 and declined with age in the two individuals for whom there were long-stored samples. From this study we conclude that a single IGF-1 determination is reliable for use in estimating disease risk after adjusting for age.

Borofsky ND, Vogelman JH, Krajcik RA, Orentreich N.

Utility of insulin-like growth factor-1 as a biomarker in epidemiological studies.

*Clinical Chemistry* 48(12):2248-2251, 2002.

## Aging and Methionine Restriction

### **Caloric Restriction vs Selective Amino Acid Restriction: Both Extend Lifespan, but...**

Forty percent calorie restriction is a well-established means of extending lifespan in rodents and other species, but the deprived research subjects are irritable. We also know that restriction of a single amino acid tryptophan extends lifespan in rodents. Elimination of the amino acid cysteine in combination with minimal methionine intake produces 40% lifespan extension while maintaining normal or enhanced levels of the antioxidant glutathione, and it does so without any restriction of food intake. We evaluated the methionine-restricted diet in several strains of rats. This diet produced extended lifespan in strains that have differing pathologic profiles of aging, and so it can be concluded that a methionine-restricted diet genuinely alters the rate of aging in rats.

Zimmerman JA, Malloy V, Krajcik R, Orentreich N.

Nutritional control of aging. (Presented at the Neuroendocrinology of Aging Conference in Austria, August 2002.)

*Experimental Gerontology* 38(1-2):47-52, 2003.

### **Restricting Dietary Methionine Quickly Benefits Glutathione Levels**

Glutathione is an important stabilizer of cell function, and decreased levels have been implicated in the aging process and in the development of numerous chronic diseases. We know that lifespan is significantly extended by a life-long diet that is either calorie-restricted or methionine-restricted. Unlike calorie restriction, a methionine-restricted diet increases glutathione levels and prevents the usual decrease of glutathione that occurs with aging. Our study found that the beneficial changes in glutathione levels were evident within only one week of starting a methionine-restricted diet. Interestingly, the increase of glutathione was in the red blood cells, not in whole plasma (where in fact the levels went down). Further, although levels of glutathione in the liver were quickly and greatly reduced, they were beneficially conserved in most non-liver tissues.

Richie JP Jr, Komninou D, Leutzinger Y, Kleinman W, Orentreich N, Malloy V, Zimmerman JA.

Tissue glutathione and cycteine levels in methionine-restricted rats.

*Nutrition* 20(9):800-805, 2004.

Collaborating Institution: American Health Foundation Cancer Center

*While the three specialized OFAS laboratories are involved in collaborative research with other organizations, each laboratory is also engaged in primary interdisciplinary research within OFAS. For example:*

This laboratory is focused on the mechanism(s) of the lifespan extension created by a full-calorie diet from which most of the essential amino acid methionine is removed (MR diet). In Fisher 344 rats, MR results in 30-40% lifespan extension, lower body weight, and fewer tumors; the rats also have lower levels of glucose, insulin, cholesterol, and triglycerides. Our questions are:

- how soon is the MR diet 'sensed'
- what metabolic adaptations are activated
- which target tissues/organs are most affected
- are there particular genes that become turned on or off
- does lifespan extension with MR involve a fundamentally different mechanism(s) than severe calorie restriction
- does lifespan extension with MR also occur in other rodents

### *Animal Biology*

This laboratory is studying a condition known as skin diabetes. The premise of this work is that not all hair loss is the common type known as androgenetic alopecia, nor is it alopecia areata. Some individuals have a type of hair loss with a reduced number of active hair follicles, either throughout the entire scalp or just on the sides. In this work, we:

- determine if the glucose content of hair shafts differs between normal and thinning areas of the scalp
- coordinate a glucose-insulin tolerance blood test with simultaneous plucking of hair follicles at selected time points to measure tissue glucose content
- determine if the blood glucose/insulin levels correlate with levels of glucose in the plucked hair follicles

### *Biochemistry*

This laboratory's research focuses on:

- effects of various non-surgical, physico/chemical mechanisms for enhancing collagen production and decreasing fat deposition in skin
- detailed exploration of the early hormonal and enzymatic metabolic effects on various tissues and organs resulting from the longevity-enhancing effect of the methionine-restricted diet
- establishing and standardizing cell-based proliferation and viability tests to assess the validity of using plasmapheresis to remove blood-borne accumulated pro-aging factor(s) or enhance the production of anti-aging factor(s)
- discovering whether the hormonal and enzymatic metabolic changes resulting from the methionine-restricted diet correlate with any anti-aging effects of plasmapheresis in the same cell-based assay

### *Cell Biology*

## Collaborations in Progress

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### Cancer

#### **Ultrapheresis® in Dogs**

This study will evaluate the use of Ultrapheresis®, a relatively new procedure that has previously been explored in human medicine, as a therapeutic treatment for cancer in dogs. Ultrapheresis attempts to reverse the immunosuppression that occurs in cancer patients by removing circulating blocking factors that interfere with the immune system.

*Collaborators: University of Pennsylvania - R. Groman, DVM, J. Baez, DVM; Animal Cancer Foundation - G.S. Post, DVM*

#### **Prostate Cancer Risk Factors**

This case-control study is examining a wide variety of potential risk factors for prostate cancer in both white and black men. The list includes growth factors, hormones, markers of inflammation, and the Metabolic Syndrome, among many others. It is hoped that such comparisons will reveal not only previously unrecognized biomarkers of risk but also why the rates of prostate cancer differ between the races.

*Collaborator: Kaiser Permanente Medical Care Program - S.K. Van den Eeden, PhD*

#### **Blood Type and Cancer Risk**

The longitudinal records of one of the largest health maintenance organizations in the world are being mined to answer the question of whether blood type (A, B, AB, O) in any way influences risk of cancer. Examined so far: breast cancer (tentatively yes) and prostate cancer (no). Future work will include lung and colon cancers.

*Collaborator: Kaiser Permanente Medical Care Program - C. Quesenberry, PhD*

### Alzheimer's Disease

#### **Proteomics Study of Alzheimer's Serum**

There is currently no routine diagnostic blood test for Alzheimer's Disease (AD); such a blood test would be enormously helpful in identifying patients for early treatment. This study will examine the proteome (the full complement of proteins) in the serum of AD patients for comparison to the serum proteome of normal controls. Proteins that are found only in patients will then be identified by mass spectroscopy and may form the basis for future diagnostic tests.

*Collaborator: University of Colorado - M. Duncan, PhD, L. Brown, PhD*

#### **Longitudinal Serum Samples to be Analyzed for Multiple Kallikreins**

The kallikreins comprise a family of serine proteases of which prostate specific antigen (PSA) is a prominent member. Another member, kallikrein 6 (K6), may be elevated in the serum of AD patients. Currently, a sensitive assay for K6 is being developed by Dr. Diamandis' laboratory. OFAS has provided longitudinal serum samples from our Blood Club Repository for the determination of the expected variability of K6 levels in normal individuals.

*Collaborator: University of Toronto - E. Diamandis, PhD*

### Alopecia Areata

#### **Alopecia Areata and Serum Antibodies**

Using the technology developed by Rules-Based Medicine (highlighted in the September issue of VitaLongevity™) wherein very small blood samples can be simultaneously measured for hundreds of biomarkers, we hope to identify clues as to the causes of this autoimmune disorder. The autoantibody profile and/or the evidence of past infection could provide leads for further study.

*Collaborator: Rules-Based Medicine, Inc. - M. Chandler, PhD, M. Spain, MD*

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### **Energy Utilization of Methionine-Restricted Rats vs. Controls**

Methionine-restricted rats consume more chow on a body weight basis than do control-fed rats without gaining weight. To address an unanswered question—where does the extra energy go?—we employed the Oxymax system to perform an energy balance study. This totally enclosed system measures and accounts for all the energy going into and coming out of the rat. The result: methionine restriction boosts energy utilization.

*Collaborator: Institute for Cancer Prevention - D. Komninou, MD, PhD*

### **The Effects of Methionine Restriction on Fat Deposition and Aging Using a Variety of Techniques**

The boost in energy utilization (see above) and early suppression of fat accumulation might be an important mediator of MR-induced lifespan extension. Preliminary examination of fat tissue from MR rats revealed that key genes involved in both fat oxidation and lifespan extension are up-regulated in MR rats. We are currently extending this work to uncover the interaction between these and other genes and how they influence the aging process.

*Collaborator: Pennington Biomedical Research Center - T. Gettys, PhD, B. Hosek, PhD*

### **DNA Microarray Studies Using Tissue from Methionine-Restricted Rats vs. Controls at Selected Time Points**

In addition to looking at specific genes in the traditional hypothesis-driven study discussed above (see Pennington), we have collected a variety of tissues from MR rats to look for global changes in gene expression over time for comparison to normally aging rats. This type of analysis using DNA microarrays generates a lot of data and provides indications of which systems in the body are most affected by an intervention, in this case methionine restriction. We will look for changes in gene expression that are similar to those occurring in calorie restriction, which also extends lifespan.

*Collaborator: LifeSpan BioSciences - G. Burmer MD, PhD*

### *Aging and Methionine Restriction (MR)*

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## **Pet Animal Serum Treasury**

The Pet Animal Serum Treasury (PAST) is a project of the Animal Cancer Foundation and OFAS. Started in 2003, it is dedicated to improving veterinary care by collecting and archiving blood (serum) specimens from diseased and healthy cats and dogs. As with the Serum Treasury maintained by OFAS and its related database maintained by Kaiser Permanente Medical Care Program, PAST will serve as a unique resource for research into the causes of or potential risk factors for cancer and other diseases in pets. It is the only resource of its kind for animals in the world. For further information, go to <[www.orentreich.org](http://www.orentreich.org)>.



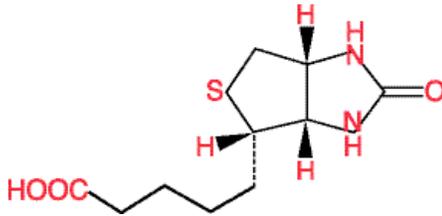
The Foundation started the quarterly VitaLongevity™ newsletter in June of this year to alert friends of OFAS to those health strategies that are valid and those that are not valid, as well as to offer new suggestions for making their lives as long and healthy as possible.

## BIOTIN (June 2004)

Brief highlights of this issue are:

- This B Vitamin is a co-factor in a number of key reactions that help convert food to energy.
- Daily intake of 0.15 to 0.3 mg is considered adequate.
- Sub-optimum biotin status might be more prevalent than is currently appreciated.
- Biotin, according to recent reports, can substantially lower fasting glucose levels in Type 2 diabetics.

The newsletter summarized: Biotin is a very safe B Vitamin. No toxicity has been reported to date at doses up to 200 mg; in fact, biotin is the only vitamin with no known toxicity. Small amounts are essential; large amounts seem very therapeutic, benefiting skin, nail, and pancreatic function.



## MAPPING THE FUTURE (September 2004)

This issue focused on the new technologies that exist for doing hundreds of tests on a single drop of blood to detect changes indicative of a broad range of diseases. The goal: to alert you and your physician to problems before they become clinically evident. Sequential testing can chart the 'velocity' of any change, which is often predictive of the severity of a disease. Other potential uses for such broad-spectrum analysis include:

- Universal disease diagnosis
  - Identification of emerging diseases, *e.g.*, SARS, West Nile Virus
  - Detection of diseases with long preclinical phases
  - Rapid diagnosis of diseases with non-specific symptoms
- Treatment and disease progression monitoring
- Drug safety and efficacy evaluation
- Blood testing for organ transplantation
- Assessment of exposure to environmental toxins

## MAPPING THE PRESENT (December 2004)

This issue will discuss the meaning and significance of basic blood tests and the importance of watching for subtle trends, even of results within the 'normal' range for age and gender.

## INFORMATION FOR DONORS

The Orentreich Foundation for the Advancement of Science, Inc. was founded in 1961. OFAS is a non-profit institution dedicated to biomedical research to prevent, halt, or reverse those disorders that decrease the quality or length of life. It is duly registered with the United States Internal Revenue Service as a 501(c)(3) Operating Private Foundation under Section 4942(j)(3).

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