December 2005

OFAS

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

This year we celebrate both the 44th anniversary of OFAS and the 20th anniversary of the consolidation of three separate research laboratories into one Biomedical Research Station in Cold Spring-on-Hudson, NY. Combining and expanding these staff and physical resources has greatly enhanced our research capabilities.

Co-Directorship: Also this year, my son, Associate Director David S. Orentreich, is stepping up his participation in OFAS to share the directorship with me. David's medical degree is from Columbia University College of Physicians and Surgeons (1980), and, as most know, he has been in full-time clinical practice with me since completing his residency at the Medical Center of Mt. Sinai School of Medicine (1984), where he is now Assistant Clinical Professor of Dermatology. David has been at my side through all the significant events—both professional and personal—of the past years, and I warmly welcome him as Co-Director.

Long-standing Research Interest: As early as 1972, the OFAS yearly Report referred to our interest in plasmapheresis as an anti-aging procedure following the leading work of Alexis Carrel. Explaining our interest, a summary of Carrel's ground-breaking research and the plasmapheresis procedure appears on page 5 herein. OFAS is currently engaged in a definitive, interdisciplinary program to explore and validate (or not) his seminal findings using today's advanced investigative techniques.

The Serum Treasury: Since founding OFAS in 1961, no achievement has been as rewarding to me as the Serum Treasury: our realization in the '80s of a research tool idealistically conceived by foresighted clinicians at Kaiser Permanente Medical Care Program in the early '60s. The Serum Treasury offers a unique opportunity to look back at stored serum from persons who later developed a disease and to do so by the rapid, cost-effective method of retrospective epidemiology. The Treasury's extraordinary utility is well-demonstrated by its having provided research girding for the 2005 Nobel Prize for the discovery of the role of *Helicobacter pylori* in gastritis and peptic ulcer disease. Using the Serum Treasury, recognized by the World Health Organization as *"one of the most valuable resources currently available in biological banking",* OFAS looks forward to many additional and exciting discoveries.

The staff and supporters of OFAS can be proud of previous and current accomplishments, independent and collaborative, and can look ahead to further achievements in the coming decades.

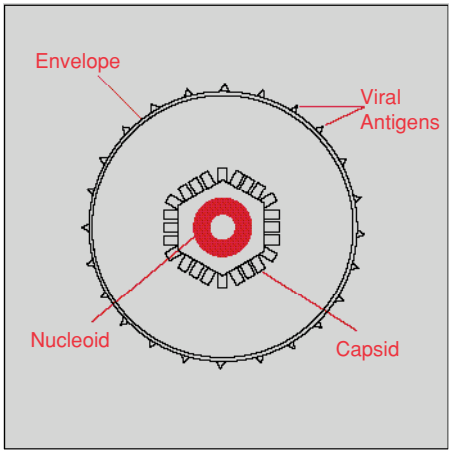
Norman Orentreich, MD, FACP
Director

Collaborations in Progress

ST *Denotes studies using the Serum Treasury.*

Viral Infection & 'Unrelated' Disease

Having had 'mono', the common EBV infection, might create a predisposition to a more serious disease such as Multiple Sclerosis and possibly non-Hodgkin's Lymphoma.



Structure of the Epstein-Barr Virus
The infectious virus particle consists of three components: a doughnut-shaped central core (Nucleoid) which contains the viral DNA in condensed form; a Capsid, which is made up of hollow, tubular protein subunits called capsomeres; and a protective Envelope incorporating Viral Antigens

With kind permission, from The Epstein-Barr Virus, *by Werner Henle, Gertrude Henle and Evelyne T Lennette. Copyright© 1979 by Scientific American, Inc. All rights reserved.*

ST **Multiple Sclerosis and Epstein-Barr Virus Infection**
Numerous studies have suggested a connection between Multiple Sclerosis (MS) and Epstein-Barr Virus (EBV), the cause of mononucleosis (aka, 'kissing disease'). Using Serum Treasury samples collected up to 30 years before the onset of MS (along with appropriate age- and sex-matched control samples), our study confirmed the association of subsequent MS with previous infection by EBV. Specifically, the study found that the relative risk of developing MS was 2 times greater if the serum sample showed a 4-fold elevation of antibodies to EBV; the study further revealed that EBV antibody levels were elevated 15-20 years before the onset of MS symptoms.
Collaborators: Kaiser Permanente Division of Research (GN DeLorenze, PhD); Harvard School of Public Health Departments of Nutrition (KL Munger, MSc) and Epidemiology (A Ascherio, MD, DrPH); Virolab, Inc. (ET Lennette, PhD); Brigham and Women's Hospital and Harvard Medical School - Channing Laboratory, Department of Medicine (A Ascherio)

ST **Non-Hodgkin's Lymphoma and Epstein-Barr Virus Infection**
Non-Hodgkin's Lymphoma (NHL) is a heterogeneous group of cancers originating from lymphoid cells. The courses vary from rapidly fatal to slowly progressing. Although the incidence increases with age, there are 8,000-10,000 new cases diagnosed each year in all age groups in the US. Although their cause(s) is unknown, there is substantial evidence suggesting that a virus is involved.

To investigate the connection between NHL and viral infection, our study used 200 Serum Treasury samples collected between 1 and 34 years before the diagnosis of NHL and 200 matched controls. All samples were assayed for antibodies to Epstein-Barr Virus (EBV). At this time, the data are being analyzed to determine any association between NHL and EBV infection by looking at the presence of EBV antibodies, the relative level of antibody response, and the time between the first detection of EBV antibodies and the onset of NHL. This is one of three collaborative studies on NHL.
Collaborators: Kaiser Permanente Division of Research (LJ Herrinton, PhD); National Cancer Institute of the National Institutes of Health (D Baris, MD, PhD)

Heart Attack & Endostatin

High levels of endostatin appear to protect against myocardial infarction in whites and Asians but not in blacks.

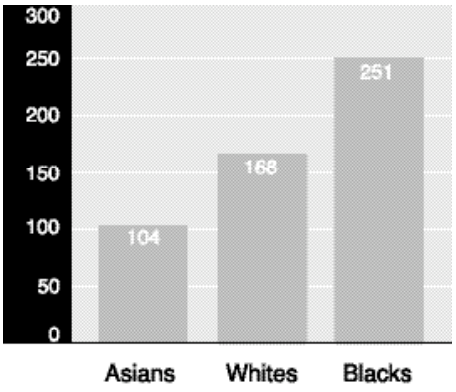
ST **Acute Myocardial Infarction and Serum Levels of Endostatin**
Not one of the statin drugs, endostatin is a protein produced by the body that reduces the proliferation of blood vessels. Because endostatin has been linked to reduced atherosclerosis development in animal models, we investigated the hypothesis that low circulating serum levels of endostatin might be associated with increased odds of acute myocardial infarction (AMI) in human beings, and whether this association varied by gender or race. We located Serum Treasury samples for 211 AMI subjects and 173 matched controls who remained free of both cardiovascular disease and cancer. Adjusting for potentially confounding risk factors (*e.g.,* body mass index, smoking), our data support the hypothesis of an inverse correlation between endostatin levels and AMI. Because this correlation was true among Asians and whites but not among blacks, further research is needed to substantiate these findings and to uncover the potential mechanisms behind the racial/ethnic differences.
Collaborators: Kaiser Permanente Division of Research (C Iribarren, MD, MPH, PhD; LJ Herrinton, PhD; JA Darbinian); CytImmune Sciences, Inc. (L Tamarkin; D Malinowski); Kaiser Permanente Oakland Medical Center, Department of Oncology (D Baer, MD)

Collaborations in Progress

ST Denotes studies using the Serum Treasury.

Cancer

ST Risk Factors for Prostate Cancer
This case-controlled study was started in 2003 to examine a wide variety of potential risk factors for prostate cancer in both white and black men. The risk factors include growth factors, hormones, markers of inflammation, and Metabolic Syndrome, among many others. Preliminary analysis suggests an increase in prostate cancer risk when markers of Insulin-like Growth Factor (IGF) are low, and that this association might be stronger in blacks than in whites. The analysis also suggests an association between the parameters of Metabolic Syndrome and increased risk of prostate cancer, and that this association might be stronger in whites than in blacks.
Collaborator: Kaiser Permanente Medical Care Program (SK Van den Eeden, PhD)



Prostate cancer cases per 100,000
Source: NCHS FASTATS (2001)

Can a highly selective filtration of plasma unblock the immune system's ability to fight cancer?

Ultrapheresis® in Dogs with Cancer
This study is designed to evaluate the use of Ultrapheresis, a relatively new procedure previously explored in experimental human medicine, as a therapeutic modality for cancer in canines. Ultrapheresis therapy attempts to reverse the immunosuppression that occurs in cancer patients; the procedure tries to remove circulating factors that block the immune system from combating cancer. Because of a major staff reorganization in the Department of Oncology at the University of Pennsylvania School of Veterinary Medicine, the planned study (10 dogs who have failed the standard therapy for their particular type of cancer or otherwise untreatable cancer) is being transferred to the Veterinary Oncology Clinic in Norwalk, CT.
Collaborator: Animal Cancer Foundation (GS Post, DVM)

Alzheimer's Disease (AD)

Proteomics and Alzheimer's Serum
There is currently no routine diagnostic blood test for Alzheimer's Disease (AD). Proteomics is the analysis of the proteome (the full complement of proteins) in serum or tissue. The primary goal of this study is to use proteomics to differentially identify serum markers from known cases of late-onset AD compared to normal controls. The ultimate goal of this project (using Research Sample Bank, Inc., sera) is to develop a specific and sensitive blood test capable of very early detection of AD and thereby to enable the earliest possible intervention.
Collaborator: University of Colorado School of Medicine, Department of Pediatrics (M Duncan, PhD; L Brown, PhD)

A blood test for the earliest hint of AD would make possible the earliest intervention.

ST Variation with Age in Serum Kallikrein-6 Levels
Kallikreins comprise a family of proteases of which Prostate Specific Antigen (PSA) is a prominent member. Some preliminary research indicates that another member, K6, might be elevated in AD. Before embarking on an extensive study of the presence of K6 in relation to AD, it would be important to know if (and how much) K6 changes with normal aging in the same individual. To this end, OFAS has provided longitudinal serum samples from our Blood Club Repository (a special segment of the Serum Treasury) to the Department of Laboratory Medicine at the University of Toronto. This lab is well-known for its research analyzing kallikreins; its analysis of the relatively rare, longitudinally collected serum samples we provided should produce important results.
Collaborator: University of Toronto, Departments of Laboratory Medicine and Pathobiology (E Diamandis, PhD)

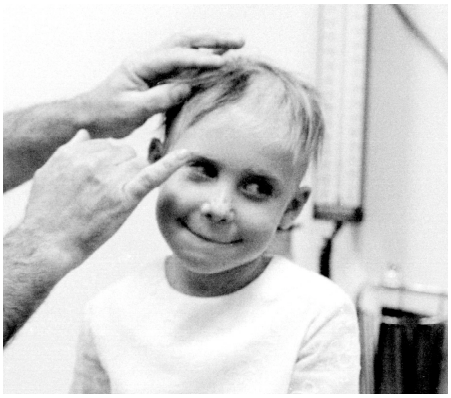
Higher blood levels of Kallikrein-6 have been associated with AD, but the association could be mere coincidence if the incidences of AD and Kallikrein-6 each increase with aging.

Collaborations in Progress

ST Denotes studies using the Serum Treasury.

Alopecia Areata (AA)

This type of hair loss is usually patchy but can be so severe as to cause loss of all hairs on the body.



Alopecia Areata and Serum Antibodies
A technology developed by Rules-Based Medicine (see VitaLongevity™, September 2004) provides a Multi-Analyte Profile (MAP) from simultaneous measurement of hundreds of biomarkers in a very small blood sample. As a pilot study, serum samples from 18 persons (including 4 children) with AA were compared with sera from 113 non-AA persons. Adults with AA were much more likely than controls to have thyroid auto-antibodies; this held to a lesser extent for the children, who had profiles quite distinct from those of adults. Further research on AA will pursue MAPing in serum from persons 18 years or less.
Collaborator: Rules-Based Medicine, Inc. (J Mapes, PhD; M Spain, MD)

Aging and Methionine Restriction (MR)

How does limiting the essential amino acid methionine to 20% of its 'required' amount extend rat lifespan by 40% and limit fat deposition?

Essential amino acids are dietary essentials because the body cannot make these building blocks of protein. They are:

- Histidine
- Isoleucine
- Leucine
- Lysine
- Methionine
- Phenylalanine
- Threonine
- Tryptophan
- Valine

Effects of Methionine Restriction on Fat Accumulation
In addition to living much longer, rats on the MR diet do not develop fat pads like rats fed standard rat chow, and this is despite MR rats eating more calories per gram of body weight. By monitoring oxygen intake, carbon dioxide output, and body temperature, this study found that feeding rats an MR diet produces a substantial and chronic increase in oxygen consumption and energy expenditure in the form of higher body temperature. It appears that limiting methionine to 20% of the 'required' dietary amount revs up the body's metabolic furnace and uses the extra energy of the higher caloric intake by dissipating it as heat; the MR diet thereby 'wastes' the extra calories and avoids depositing them as fat pads. In the real world of rats in the wild, this phenomenon would be a severe handicap because it is an inefficient, wasteful use of calories that would be better stored as sources of sustenance in lean times. For research in aging, however, the MR diet provides an ideal model to understand how metabolic inefficiency delays aging.
Collaborator: Pennington Biomedical Research Center (T Gettys, PhD; B Hasek, PhD)

MR in Human Subjects
Currently in the planning stage, this study would impose short-term (4 month) MR on obese Metabolic Syndrome subjects and evaluate changes in body composition and insulin action by a variety of methods including blood tests, physicals, indirect-calorimetry, multi-slice CAT scans, energy-balance assays, oral glucose tolerance tests, and euglycemic-hyperinsulinemic clamp studies.
Collaborators: Pennington Biomedical Research Center (F Greenway, MD; T Gettys, PhD); Abbott Laboratories, Ross Products Division (B Marriage, PhD, RD)

MR and Gene Expression in Specific Tissues
For this study 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, which seek to quantify the level of expression of each active gene within a tissue or cell, generates abundant data and provides indications of which systems in the body are most affected by an intervention, in this case MR. The study will look for specific changes in gene expression that are similar to those occurring in calorie-restricted rats, which, like MR rats, show extended lifespans.
Collaborator: LifeSpan BioSciences, Inc. (G Burmer, MD, PhD)

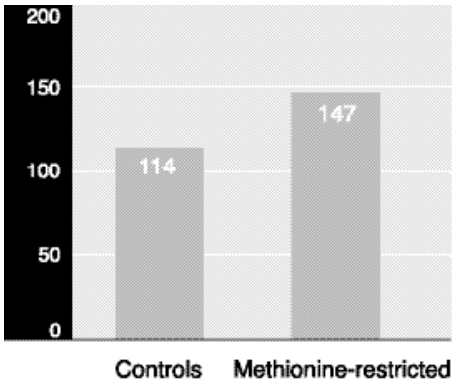
While the three specialized OFAS laboratories are involved in collaborative research with other organizations, each laboratory is also engaged in primary interdisciplinary research within OFAS. For example, in addition to other projects, the laboratories currently share a focus on aging.

Methionine Restriction (MR)
Studies at OFAS have established that lifespan extension can be achieved in the rat by simply reducing one dietary essential amino acid (methionine) and that the attained longevity is comparable to that achieved by severe calorie restriction. To establish that MR has broad anti-aging significance and is not a phenomenon unique to the rat, this laboratory is evaluating:

- MR's anti-aging effect(s) in mice, which appear to need even less methionine than rats
- Metabolic effects of a β-adrenergic-receptor blocker of the sympathetic nervous system on adipose tissue formation in rats on the MR diet
- Growth effects of repletion with Insulin-like Growth Factor-1 on MR rats
- Early hormonal changes associated with MR in rats

Plasmapheresis
While the Cell Biology Laboratory will use *in vitro* methods to demonstrate anti-aging effects of plasmapheresis (see below), the Animal Biology Laboratory will evaluate classic *in vivo* parameters for the assessment of aging. To be selected, parameters must be able to show potential changes either within the 3-month period of weekly plasmapheresis or the 3 months post-plasmapheresis. Classic *in vivo* aging criteria include: wound healing, body weight, food intake, cataract formation/progression, nail growth rate, nail plate cell morphology, tail collagen strength, proteinuria, sexual behavior, levels of spontaneous activity, glucose tolerance, cognitive function, skin *vs* body temperature, responses to various stresses, muscle strength, disease incidence, immune system function, and changes in sensory systems.

Animal Biology



Median lifespan in weeks of Fischer 344 male rats

What are the best, rapid ways to evaluate an aging treatment?

AGING: Plasmapheresis and Alexis Carrel

The plasmapheresis procedure entails withdrawing a specified amount of whole blood (as much as one-third of total volume) from a human being or animal. The whole blood is centrifuged to separate the fluid plasma from cellular components. The plasma is discarded or used for research; the cellular components (red blood cells, platelets) are resuspended in a plasma-replacement fluid and reinfused into the subject.

Alexis Carrel (1873-1944) was a medical scientist of diverse achievements. His accomplishments in vascular surgery and organ transplantation earned him the Nobel Prize in 1912. He also was deeply interested in aging.

As hypothesized by Carrel, plasma from old and young animals would differ and this difference would reflect itself in the ability of cells to proliferate. While at the Rockefeller Institute for Medical Research, he tested his hypothesis using advanced tissue culture techniques of the time (1920-40, some of which he developed) to assess the effects of serum from donors of different ages on cell proliferation. Carrel found that aging produced a relative accumulation of 'inhibitor' substances and diminution of 'accelerator' substances in plasma. Carrel pursued his findings by using plasmapheresis to remove the accumulated inhibitor substances and stimulate production of accelerator substances; the experimental treatment resulted in clinical signs of rejuvenation in plasmapheresed animals.

Most of Carrel's work was published in the *Journal of Experimental Medicine*. Though these papers provide more than adequate 'proof of concept' for his hypothesis of extracellular, serum-based aging factors, his seminal findings have never been systematically replicated.

In-house Research

Biochemistry Laboratory

Can the aging effect of oxidative stress be measurably ameliorated?

Free Radicals
The ability of serum to remove oxygen radicals might be a good measure of biologic age because younger animals are more capable of combating these highly reactive and potentially damaging molecules. Because they can neutralize oxygen radicals, this laboratory is measuring free thiol (sulfur-hydrogen) groups *vs* disulphide (sulfur-sulfur) groups as an indicator of biologic age. We are assaying sera from rats and human beings with the expectation that free thiols will be higher in the young and that interventions such as plasmapheresis or MR will, respectively, reduce or prevent any excessive accumulation of disulfide groups in the old.

Cell Biology Laboratory

How does a diet limited to 20% of the 'required' amount of the essential amino acid methionine limit obesity?

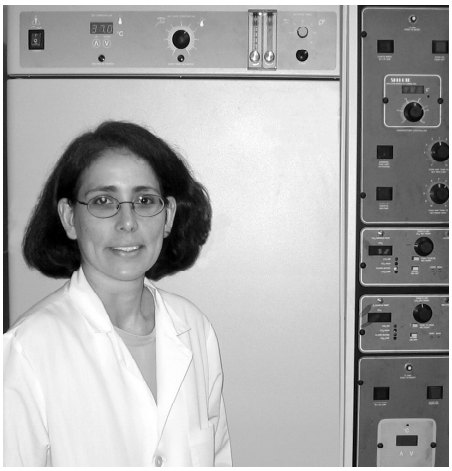
Methionine Restriction (MR)
MR in rats extends lifespan and prevents the age-associated build up of adipose tissue. In human beings, cortisol (one of the glucocorticoids) has been implicated in the transition of pre-adipocytes to mature adipocytes. In rats, the glucocorticoid that modulates adipose tissue formation appears to be corticosterone.

Using a variety of *in vitro* techniques, the Cell Biology Laboratory is assessing the effect of MR on the activity and expression levels of the enzyme that adjusts corticosterone levels in rats to determine whether either or both of these account for MR rats avoiding obesity.

Do the effects of plasma on cell growth differ with age and anti-aging treatment?

Plasmapheresis
Using today's sophisticated techniques and specialized cell lines while building on the work of prominent researchers in the characterization of aging cells, the Cell Biology Laboratory is reviewing and validating the findings of Carrel (see page 5) using plasma from rats of different ages. The project will be extended to study plasma from mature rats that have been plasmapheresed as well as plasma from chronologically mature but biologically young rats that have been on the MR diet.

New Head of Cell Biology Laboratory



Carmen E. Perrone, PhD, received her doctorate in Cellular Biology from the University of California, Los Angeles (UCLA) and conducted post-doctoral research at Brown University and the American Health Foundation. At Brown she was involved in studies geared to understanding skeletal muscle atrophy. At American Health she used cell biology techniques in her toxicology work on the lipid-lowering agents classified as peroxisome proliferators. In 2000 she was appointed Research Assistant Professor in Pathology at the New York Medical College (NYMC) where she worked in the areas of toxicology, cancer, and pulmonary hypertension.

Dr. Perrone joined OFAS in August 2005 as Head of the Cell Biology Laboratory. She also holds adjunct professor positions in Pathology at NYMC and in Biology at Manhattanville College. She serves on the editorial review board for the *Journal of Photochemistry and Photobiology.* In addition, she is a member of the American Association for the Advancement of Science, the American Association for Cancer Research, and the Federal Insecticide, Fungicide and Rodenticide Act Scientific Advisory Panel.

OFAS started the quarterly VitaLongevity newsletter in June 2004 to alert friends 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.

DHEA and DHEAS (December 2005)
Research at OFAS established the normal human blood levels of DHEAS and its 80% drop between about 25 and 70 years of age. DHEAS is a prohormone that tissues selectively make into active hormones (androgens and estrogens); it is not a 'muscle-building steroid'. Safety and replacement therapy guided by blood tests are discussed.

VITAMIN D (September 2005)
Adequate Vitamin D (from supplements, diet, or sunlight) is important not only for bones but also for blood pressure, glucose levels, and immune system function; it might even prevent some cancers. Adequate calcium is an important component of Vitamin D metabolism.

GLUCOSE TOXICITY (June 2005)
This issue stressed the importance of keeping blood glucose levels low throughout the day. Glucose reacts with proteins, ultimately forming Advanced Glycation End-products (AGEs) that are detrimental to many tissues and lead to premature aging.

CALORIE RESTRICTION and LIFESPAN EXTENSION (March 2005)
Calorie restriction (CR) plays a unique role in the field of aging research. CR improves insulin action, possibly the reason for its life-extending properties. Nutritional approaches and supplements to achieve some of the benefits of CR without cutting back food consumption are discussed.

MAPPING THE PRESENT (December 2004)
This issue discussed 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.

MAPPING THE FUTURE (September 2004)
The focus of this issue was the new technologies that exist for conducting hundreds of tests on a single drop of blood that can 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.

BIOTIN (June 2004)
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 therapeutic, benefiting skin, nail, and pancreatic function.

Copies of past issues are available upon request or can be downloaded from our website at <www.orentreich.org>.

Publications

ST Denotes studies using the Serum Treasury.

ST de Martel C, Llosa AE, Farr AM, Friedman GD, Vogelman JH, Orentreich N, Corley DA, Parsonnet J
Helicobacter pylori infection and the risk of development of esophageal adenocarcinoma.
Journal of Infectious Diseases (2005) 191(5):761-7

Malloy VL, Krajcik RA, Bailey SJ, Hristopoulos G, Plummer JD, Orentreich N
Methionine restriction decreases visceral fat mass and preserves insulin action in aging male Fischer 344 rats independent of energy restriction.
Submitted

ST DeLorenze GN, Minger KL, Lennette ET, Orentreich N, Vogelman JH, Ascherio A
EBV and MS: evidence of association from a prospective study of patients in a large integrated health plan.
Submitted

ST Iribarren C, Herrinton LJ, Darbinian JA, Tamarkin L, Malinowski D, Vogelman JH, Orentreich N, Baer D
The association between serum endostatin, an endogenous angiogenesis inhibitor, and acute myocardial infarction differs by race: a case-control study.
Submitted

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 pets, typically 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.

Currently containing over 500 samples, PAST is the only resource of its kind for animals in the world. As with the OFAS-Kaiser Serum Treasury, the samples will be used to see if any serum factors are present in pets that can predict or identify diseases in their earliest stages. The database contains information on the pet, its environment and eating habits, and veterinarian reports pertaining to its condition and treatments; over 40 different parameters are collected for each animal, and any combination of data can be searched for and retrieved in seconds. For further information, go to <www.orentreich.org>.

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).

No accomplishment of OFAS is possible without your encouragement and generous support. Your tax-deductible contribution should be mailed to:

Orentreich Foundation for the Advancement of Science, Inc
910 Fifth Avenue
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