

Dutch Study Phase X (Phases I, II, III, IV, V, VI, VII, VIII IX & Literature Citations available on request)

This is the tenth and final part of a personal chronology of my experience implementing the conclusions of the Rotterdam Study (New England Journal of Medicine Nov. 22, 2001). The chronology was started as a memo to my younger brothers and sisters. We have active sporadic Alzheimer's disease in our family history. Our father, his sister and with less assurance, their mother, became clinical victims at their ages of 74-75. Both maternal and fraternal aunts and uncles of my father had AD by 75 years of age. The NIH estimates that this genetic history projects to a 25% risk of AD. The incubation period of AD is likely long, between 4 to eight years, before a clinical diagnosis can be made. I am intent on dodging that bullet and I am pulling out all the stops in that regard. With perseverance I have dodged prostate and colon cancer bullets earlier in my life, and reaching 69 this is just another one. This Alzheimer's challenge is embraced with the joy of discovery and without fear.

Previous reports have illustrated an evolving AD Prevention program, anchored on the use of chronic levels of NSAIDs as reported by the Erasmus Medical Center (EMC) in their Rotterdam Prospective Epidemiological Study. In the last several of these Dutch Study Reports are revealed the intensifying use of anti-oxidant vitamins E & C, the B vitamins, Omega 3 fatty acids from fish, neuron stimulating chemical, Acetyl-L-Carnitine, and a five year history of anti-hypertension therapy. Statins had not been used because LDL is ultra low to start and this was a characteristic of my father's (AD victim) blood chemistry, and also a younger brother's (symptom free). I came to believe the statins deserved a place in this program. The older and proven drugs Mevacor and Zocor are off patent in several countries. Zocor is now an OTC drug in England. A Low dose statin regimen was put in place in early June, starting with low dose 10 mg/day simvastatin (Zocor) and changing to medium dose 20 mg/day lovastatin (Mevacor) now in the 177 th day.

Turmeric dosing had been slowly increased to 135 mg/kg-day from June 1, 2004. The curcumin assay of the spice (Raja Foods Inc.) is 2.8%. Dosing on a curcumin basis was now 3.8 mg/kg-day, about three quarters (75%) the metabolic equivalent of 25 mg/kg-day shown to be effective in mice. The use of the whole spice was curtailed 9/23/04 when routine fasting glucose readings began to climb towards 150 mg/dL. The three days per week a 500 mg capsule of concentrated curcumin (WWW.CURMAX.COM) was expanded to seven (7) days for a dosage of 4.5 mg/kg-day from that source.

[THE CHRONOLOGY RESUMES]

December 2, 2004 Update at 36 Months:

Today completes 36 months of my Alzheimer's disease Prevention Program (ADPP), following the results of the Rotterdam Study disclosed in NEJM Nov. 21, 2001. NSAID's use has been at 33-80% of the Study's Defined Daily Dose (DDD) using Ibuprofen (1000 days) and Naproxen (82 days). The program started with IBP @33→ 50% DDD, and then switched for a short period to Naproxen @ 80% DDD. The switch proceeded until July 25th 2002 (eight months into the program) when it was learned that select NSAIDs (Nature 414, Nov. 8, 2001; Koo et al) had been correlated by the Rotterdam Team at Erasmus Medical Center (EMC) and found to contribute disproportionately to their prior statistical correlations. IBP, one of the three NSAIDs, which inhibit a protein-slicing enzyme, which produces a 42 A-Beta (A β) amyloid, an insoluble polypeptide shown to clump into plaque aggregates. Subsequently all NSAID use was returned to Ibuprofen @ 50→67% DDD. Ibuprofen use had been predominantly at 600 mg/day (50% DDD) except for excursions of 20-38 days at 800 mg/day (67% DDD). The excursions had been terminated for Tinnitus once, and for foreign travels or extensive domestic trips. The prior periods approximate days at 800 mg were 100.

The immediate period reported on here is the 90 days leading up to the 36-month program milestone. The period was marked by continuance of an intensification of the basic program following verification of having inherited an e3, e4 ApoE genotype. The intensification amounted to increasing Ibuprofen to 800 mg/day, now into its 377nd consecutive day. (a total of 477 days since program start in November 2001).

Other aspects of the program were intensified earlier as well. Vitamin E (natural) to 1200 IU/day from 800 (synthetic). Continuance of 1000 mg/day of Vitamin C. Folic acid increased from 600 mcg/day to 1000, and in the last period raised to 1370. B12 increased from 30 mcg/day to 300 mcg/day and in the last period to 525. B6 dropped from 30 mg/day to 22 mg/day. Fish oils increased from 2 grams/day to 6 grams/day using 5 gm/day of Cod Liver Oil as the additional source of DHA/EPA, with its generous amounts of vitamin D. Expensive Acetyl-L-Carnitine has been continued but since March 2004 at a reduced 300 mg/day level from that used for one year at 500 mg/day.

Blood Analysis at 325 days of ADPP intensification

On October 15, 2004 I had a comprehensive blood assay to reflect 34.5 months of the ADPP experience. The focus was on kidney and liver function through a complete metabolic panel, a systemic inflammation assessment through total Homocysteine, a broad measure of glucose level through a glycolated hemoglobin assay, and a lipid panel to access the effects of low dose Statin and Niacin therapy. These are captured in the table below and compared with earlier tests throughout the year long ADPP intensification.

The 46 week program intensification displays stability in liver and kidney function parameters, and these parameters also are very close in the values at baseline 1080 days back from the last determinations. Concern over glucose levels has not gone away but the last determination at 118 mg/dL is almost exactly the equivalent (117) of the HbA1c test at 5.5% which represents an integrated average of glucose levels over the prior 8 weeks. Together with a glucose tolerance test performed November 2002 (ADPP program day 370), the possibility of existing diabetes has been ruled out but the susceptibility is plain. Preliminary prevention strategies, employing cinnamon concentrate, are in place starting 11/08/04

The effect of the statin/niacin usage is evident in total cholesterol lowering of 20%, enabled through LDL lowering of 50%, and triglycerides lowering of 25% while increasing HDL by 10% (the Accumed determination judged in error because of a long history of HDL ca. 28-29 mg/dL). This level of LDL is so unusually low we have not been able to make a judgment of its implications, bad or good.

The greatest satisfaction is the sustained low level of Homocysteine. In fact the level dropped from 9.9 to 8.8 $\mu\text{mol/L}$, quite possibly from folic acid, B12, and the statin all increased or introduced in the last 20 weeks. The low Homocysteine level, 2.7 points below the average for this age, and the recent trend lower, is strong assurance that brain atrophication is not occurring. MRI detectable brain atrophication precedes clinical AD by 4-11 years, so this is the best assurance I now have that the prevention program has worked to date.

Hypertension therapy preceded the ADPP. I was in classic denial about a slow, decade long, rise in blood pressure as measured and noted by my physician and more frequently at each BCNJ donation. After a business trip to Botswana in late 1996 I was unable to donate platelets for one year (1997) during which the blood pressure continued its inexorable rise, but not noticed. Finally I was hearing it audibly while lying in bed. I associated this with suspected sleep apnea and worried whether my gasping for air (commented on for by my

partner of then 42 years) was the cause of a thickening heart muscle wall and high blood pressure evidencing as an audible pounding. The denial ran deep including the surreptitious scheduling of a sleep clinic session at a local hospital. The whole episode is almost embarrassing to recall as an example of how far one can go in an absurd attempt to avoid facing the facts. The cat got out of the bag, my physician was told of the results (no apnea but airway problems) which he corrected with a breath right strip, and got me on blood pressure medication.

With some experimentation, the medication regime has been optimized to a β blocker and diuretic in the AM, and a ACE inhibitor in the evening. Blood pressure is maintained at 125 ± 10 systolic, 75 ± 5 diastolic. Home monitoring weekly compliments the BCNJ measurements monthly and physician visits quarterly. The fit of this with AD prevention has been only slowly appreciated. The bernouliists see this as fundamental to perfusion of blood to all sections of the brain. A wonderful book published by McGraw Hill in 2003, *The Memory Cure*, by Majid Fotuhi, MD, PhD of Johns Hopkins, captures the central position of controlling hypertension in AD prevention when he places this as Step 1 in his ten step program of memory retention. This text is in my opinion the clearest outline of the measures one can take to avoid Alzheimer's disease. It came along too late to define my program but I am gratified that most of the initiatives taken, inspired by the post 1997 research progress, have placed me in good alignment with its teachings.

Later I will outline a disease model which I have pieced together from all the AD reading and communicating with AD researchers. I call this 'Taking on Alzheimer's disease', and its perspective is modeled after Andrew Groves article in *Fortune Magazine* when he addressed his own case of prostate cancer. I like to feel I have a professional kinship with Dr. Groves a fellow chemical engineer. The recent work of Takashi Ohruai stimulated the model's formation which already had been germinating in the subconscious.

Blood Testing in the Intensification Period

Days ADPP intensification following November 20, 2003

Parameter	0 days	52 days	67 days	90 days	95 days	120 days	150 days	182 days	325 days
Glucose, mg/dL		138 ¹		101 ²	117			132	118
Calcium, mg/dL		9.8			10.6			10.2	9.5
Blood Urea Nitrogen mg/dL		20			17			14	16
Creatinine, mg/dL		1.3			1.3			1.4	1.3
BUN/Creatinine ratio		15.4			13.1			10	12.3
Albumin, g/dL					4.6			4.7	4.3
Albumin/Globulin ratio					1.4			1.4	1.2
Total protein, g/dL					8.0			8.1	8.0
Platelets cells/mL ³	188,000		261,000	217,000		215,000	212,000	210,000/204,000	229,000 ⁴
Hemoglobin, g/dL	14.2		15.5	13.9		14	14.4	13.8 /15.3	13.6
White Cell Count, cells/mL	8,900		8,500	6,100		7,100	6,800	6,700/7500	7,900
HDL, mg/dL				31				22	32
LDL, mg/dL								46	23
Triglyceride, mg/dL								337	246
Total Cholesterol, mg/dL ⁵	125		150	127 ⁶		129	133	131 /135	104 ⁷
Bilirubin, mg/dL					0.66			0.6	0.77
Alkaline Phosphatase, U/L					54			51	53
AST, U/L					25			27	24
ALT, U/L					35			31	30
C- reactive protein, mg/L					1.1				---
Homocysteine, μ mol/L					9.9				8.8
HbA1c %									5.5 ⁸
Test Location	BCNJ	Quest	BCNJ	BCNJ/MMH ⁹	Quest	BCNJ	BCNJ ¹⁰	BCNJ/Accumed ¹¹	Quest

¹ Meal taken incorrectly just before testing. Was unawares that basic metabolism included glucose.

² 2.0 hours after four cookies and two glasses of apple juice at BCNJ donation site.

³ Three years, nine months of BCNJ donations (31), thru donation 73 with average platelet count of 222,000 \pm 23,000 cells/mL

⁴ Avg. Platelets, Hemoglobin, White Cell Count (immediately below) from BCNJ donations 68,69,70,71 6/19 thru 9/20, 2004

⁵ Four years, of BCNJ donations (29) thru donation No. 67 with average total cholesterol at 131 \pm 7 mg/dL

⁶ Average of two tests, one at each institution, \pm 1.0 mg/dL

⁷ After 130 days of low dose Statins were added to ADPP: 10 mg Simvastatin, then 82 days 20mg Lovastatin.

⁸ This glycolated hemoglobin A1C assay reflects average glucose over prior 8 weeks. < 6% for non diabetics is normal.

⁹ Morristown Memorial Hospital community heart clinic. Piggybacking on wife's appointment after BCNJ No.64

¹⁰ Blood Center of New Jersey platelet donation No. 66

¹¹ Where two values are presented these are from BCNJ/Accumed Diagnostic Laboratory respectively. BCNJ donation 67

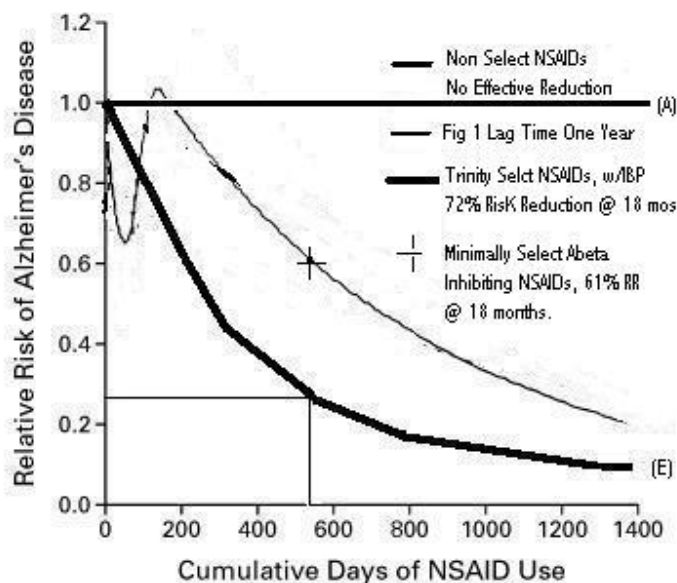
The NSAID correlation from the Rotterdam Study

The family of curves below represents my current assessment of the Rotterdam Study's re-analysis. Study (NEJM Nov. 2001) Figure 1 is amended by their own disclosure at Stockholm July 25, 2002 with the 18th month risk reduction attribution to the Trinity of NSAIDs (Ibuprofen, Indomethacin, Sulindac) shown by the Koo/Golde team's to inhibit Abeta 42 enzyme activity. The course of the heavy line is arbitrary except for the origin and 18th month risk reduction Posted by the Erasmus Medical Center for this group. The light curve (original RS finding) now represents the risk reduction of the mildly A β -42 inhibiting NSAIDs (diclofenac and piroxicam) disclosed in a second report by the Koo/Golde teams, and the privileged information I received on the "neutrality" of diclofenac for the whole of the NSAID usage. Diclofenac's effects could be left in or discarded and the risk reduction would not change from the original Figure 1 curve. Diclofenac was the most highly used NSAID (total of 43% of prescriptions, 36% of usage duration) by Rotterdam Study participants and their respective physicians.

To get this weighted conformance of the mildly inhibiting NSAIDs to the original curve, you need to assume that those NSAIDs which show no in-vitro or in-vivo inhibition (like naproxen, 17% of Study weighting) had no contribution to AD risk reduction (flat line A).

From the curve below, at ca. 1000 days of NSAID use, the Alzheimer's disease risk is now 15% of what it would have been without the use of A β 42 inhibiting NSAID.

The response to ibuprofen and nutritional supplement intensification has been so favorable that all earlier thoughts of switching NSAIDs will be postponed going forward, allowing for the incremental participation of multifunctional curcumin and the continuation of statin usage via Lovastatin.



Current LGN Rotterdam Study Re-analysis

(A= Assumed) (E= Estimated)

The completion of 36 months of this aggressive prevention program without medical complications has been very satisfying. The guidance and co-operation of my physician consultant, and many medical and scientific investigators has been deeply appreciated.

Editorial Opinion

Some of the criticisms made in my last report (DS IX) related to the impressions of the 9th International Conference on Alzheimer's disease and Related Disorders, in Philadelphia, apparently were not well received. The silence from researchers whose work was admired and where added information was sought, is puzzling. This is not universal. The Rotterdam Study representative at Philadelphia later explained that their work will be wrapped up in a major update at the end of 2004 and we can expect to see the analyzed results in mid to late 2005. This is encouraging to hear from what is in my opinion the best medical epidemiological team in the world.

I may have stepped on some sensitive toes in characterizing the 12% annual trial drop out rate of the Mayo Clinic managed donepezil/vitamin E/placebo AD progression trial as excessive and demanding of a thorough explanation, not given in Philadelphia. This is a major problem in North American trials which I do not believe can be corrected with statistical methodologies. A classic example has exposed itself in the recent Vioxx recall. The so called trial (VIGOR) which supposedly showed that Vioxx had a substantial cardiovascular risk when compared to naproxen when these two NSAID's were compared in a Gastro-Intestinal incident face off had a 29% drop out rate in a one year, 9 month median patient treatment trial. This is an astonishing drop out rate of 38% per year. I had used the results of that trial to explain and justify the extreme care and monitoring I was employing with chronic NSAID use (Dutch Study Report-V). I was puzzled then by the GI risk exposure data presented in figure 1 of the Vigor Study paper and had written to the author for an explanation of the declining numbers of apparent participants. There was no reply from the study leader. Now I understand better why. I believe that any study financed with public money (VIGOR was not) that experiences a drop out rate in excess of 6%/year (deaths and residence moves away from the research centers being exceptions) should result in a cut-off of federal support for other trials by any of the leaders of the mismanaged effort for 5 years. There is just no reliable scientific information that can result from medical trials with higher drop out rates.

Merck paid for the VIGOR trial. It seems to me that they are the big loser from the professional mismanagement of a trial conducted with a unforgivable drop out rate.

Latest Outstanding Research Findings

A period of encouraging research was reported on this Fall. There were two outstanding examples. The work of the Frank LaFerla team at University of California at Irvine (Neuron V.43 Aug.5 2004) with simultaneously proving the amyloid cascade hypothesis and indirectly demonstrating the power the β Amyloid vaccination principle which is the north star of a Alzheimer's disease cure. The other is the insightful work of the Takashi Ohnishi team at Tohoku University (Neurology v.63 (7) 2004 & Jol. Amer. Geriatric Soc. V 52,2004), Japan

combining a large epidemiological study and a small clinical trial of select classes of hypertension drugs which influence the incidence of AD and the progression of cognitive decline in AD victims. What sets these results apart from the regular baby steps of the AD research community is the sweep of the insights one gets from the work as each builds on the pioneering work by so many others.

LaFerla's team injected amyloid directly into the one side of the hippocampus of triple transgenic mice (mice which develop both plaque and neurofibrillary tangles) and show that the antibodies to the amyloid clear the plaque build up rapidly and then, for younger mice, slowly clear the tangles. If the mice are older the plaque clears but the more mature tangles do not. For younger mice, after a time the plaque reoccurs, and after a further interval the tangles again form. The main lesson is the formation sequence of the respective clearance and reoccurrence. Other clearance modes and agents are tried. All in all this is convincing evidence that plaque formation precedes and causes tangles and neuron death in human AD. The work also suggests that timely amyloid vaccination can clear both plaque (already demonstrated in the Elan Pharmaceutical trial) and tangles in humans. Vaccinations, before tangles have matured through extensive phosphorylation, may restore cognition in mild cognitive impairment (MCI) stages.

The magic number on the conversion of mice ages and plaque clearing and reoccurrence intervals is 50 to compare with human ages and intervals.

Ohri's team compared blood brain barrier penetrating Angiotensin Converting Enzyme (ACE) inhibiting antihypertension drugs with non penetrating ACE inhibitors, calcium channel blockers, β blockers, and diuretics, all antihypertensives, for their effect at lowering the incidence of AD in a large (4100 patient) 8 patient-year long prospective epidemiological study. The BBB penetrating (BBBp) ACE's were found to have a 75% lower incidence of AD.

The same BBBp ACE's and non BBB penetrating (BBBnp) Ace's and the calcium channel blockers were used on AD patients with moderate disease in a one year trial using the Mini Mental State Exam (MMSE) to follow disease progression in the three groups of 51-58 nursing home patients each. The BBBp ACE users had only nominal cognitive decline whereas the other two groups' cognitive decline was severe but still less than that expected from the natural history of the disease, without intervention. The clinical trial progression study confirmed the BBB findings of the prospective epidemiological study respecting ACE inhibition benefits in the blood nourishing capillaries of the brain cognition centers.

Fortunately the ACE inhibitor I have been using is mainly chemically identical to one of the study BBBp drugs, and has a high lipophilic and low hydrophilic characteristic in relation to the BBBnp reference drug. Data furnished by the drug manufacturer comparing brain converting enzyme activity, in rat brains, of this drug with the Ohuri study ACE's was significantly (3X) greater, giving me some assurance that my ACE would have BBBp characteristics.

Taking on Alzheimer's disease Through Prevention

Introduction

It would be a unsatisfying experience to have immersed yourself for three years in a subject where you have a probability for a serious pending consequence, and not have developed a working model or handle on the subject. A career in problem solving has compelled me to "solve the problem" then later test the solution for its sanity, over time. AD prevention will be no exception. I now know it all. I have the solution!

There comes a time in the intense study of any subject when you come to the conclusion that you 'know it all'. I have been there many times in, crystallization, evaporation, polymerization electrolysis of brine, starch liquefaction, sugar fermentation, piping design & fabrication, and mega project construction contracting. In each of these mastered fields I have learned again and again that "it's what you learn after you know it all...that counts". That said, we should not let that truism or the demands of empirical scientific rigor develop into rigor mortis.

The model's development will make use of the concept of the Blood Brain Barrier (BBB). Candidly, at the outset my idea of this barrier was that of a supermarket shrink wrap layer of micro porous polyethylene enveloping the brain. Barriers like this are used industrially for isotope, and air component, separations driven by pressure differentials, barometric and osmotic. More sophisticated barriers with sandwiched hydrophilic and hydrophobic layers are used to separate ions driven by electric potentials. It is from that background that I started to think. Size seemed the most important, molecular weight and configuration followed. Some gentle souls advised that it was orders of magnitude more complex than that. So "you think and you learn. You learn." in the words of Leonard Virilli my old barber/gardening/financial consultant. The shrink wrap vision has yielded to the reality of thousands of miles of arterial vessels, and capillaries, and ball rooms of interfacial areas. Here is the result of that thinking process, addressed to the realities of anatomy.

Details of the Model

Everybody's blood brain barrier (BBB) gets less flexible and transmissible over time to solute molecules sub micron and micron size particles.

Persons with APOE-4 genotype have tighter BBB structures and they tighten further, and earlier. They allow less in and more importantly less out (clearance). Technically this is a dysfunctional endothelium. The dysfunctionality most probably stems from errors in the repair and renewal of the endothelial cells lining the micro vascular system of the brain.

Amyloid beta oligomers and higher polymers which freely passed out by diffusion below age 55, have more difficulty passing each year, peaking at 65 for E4 homozygous at 75 for E4 heterozygote, age 85 for homo E3 combinations, and later yet with hetero E2.

A β plaque, depositing as oligomer concentrations rise, cause inflammation as macrophages spring into action to remove plaque by depolymerizing. In creating smaller oligomers, with no way out, the process snowballs. It should be noted that we are talking about minute quantities of plaque, occurring over decades, perhaps 4 milligrams total deposit mass spread throughout the brain when the victim has severe disease. These estimates are based on anatomically sampled brain sections with inferential gravimetric determinations. These are complimented with material balances of A β in Cerebral Spinal Fluid (CSF) comparing higher (unrestricted) concentrations in healthy controls with 60% lower (restricted by BBB) values in AD victims, and knowing average values of the CSF traffic from brain skull to spinal column, about 500 cc/day (See Neils Andreasen paper June 1999, JAMA's Archives of Neurology).

Cholesterol feeds the A β production process. By lowering LDL in the blood stream, LDL, high naturally in the brain, is lowered as its diffusion from brain to blood is promoted. With high LDL in the blood (untreated hypercholesteremia), LDL is diffusing slowly into the brain. Using a BBB transiting statin lowers LDL in blood and perhaps in CSF and in intracellular domains.

Angiotensin Converting Enzyme (ACE) inhibitors for hypertension are effective in systemic vascular dilation in the gross arterial network, but those which transit the BBB especially

sustain cognition by reducing arterial tension and maintaining perfusion in blood brain tributaries and capillaries, and uniform distribution of CSF efflux from ventricles.

All Cox-1 NSAIDs inhibit microphage inflammatory response to A β plaque deposition, slowing the cascade. A β -42 inhibiting Cox-1 NSAIDs selectively reduce the production of this A β plaque and thus natural clearance past the BBB tends to keep up with the reduced production of the agglomerating 1-42 length polypeptide.

Antioxidants, born in blood circulation, tend to keep the BBB flexible and porous, thus keeping the clearance function alive. Endothelium repair is facilitated by essential Omega 3 fatty acids. Important members of the B vitamin family dampen systemic inflammation occasioned by repair and A β depolymerization. Folate and B12 can even checkmate atrofication of brain structures and thus sustain alternative cognitive regions in the presence of extensive AD pathology.

This Alzheimer's is a disease which is theoretically measurable as the ΔP between brain cavity and arterial system for the flow of CSF A β out of the extra cellular brain system into the body's natural elimination systems.

Synopsis

While I will continue with a maintenance level of the program this will be the last regular report. I am capable only of a sustained obsession of no longer than three years. This has been the experience over the years with subjects as diverse as breeding and training bird dogs, growing asparagus, measuring heat loss in residential buildings, practicing the work physiology of running and aerobics with the Dr. Kenneth Cooper methods, and understanding prostate cancer. It may have started with the Charles Atlas body building program through "Dynamic Tension" when I was about 13, and then finding I could not pay the bills which were accumulating and discovered by my Mother. When I think back of that stinging letter she wrote to "Mr. Atlas" to stop sending the bills, I used to cringe with embarrassment. It all seems so funny now and perhaps this was the start of a lifelong pursuit of obsessions.

Larry G. Nault
December 2, 2004

