MACUB

IN VIVO

The Publication of the Metropolitan Association of College and University Biologists

Fall 2011 Volume 33, Issue 1

44th ANNUAL MACUB CONFERENCE

Saturday, October 29, 2011

at

SETON HALL UNIVERSITY

South Orange, New Jersey

CONFERENCE THEME

Microbes: Tiny But Not Insignificant

KEYNOTE ADDRESSES

Bonnie Bassler

How Bacteria Talk to Each Other

Martin J. Blaser

A Mixed Bag:
Bacteria That Colonize Humans

Instructions for Authors

IN VIVO is published three times yearly during the Fall, Winter, and Spring. Original research articles in the field of biology in addition to original articles of general interest to faculty and students may be submitted to the editor to be considered for publication. Manuscripts can be in the form of a) full length manuscripts, b) mini-reviews or c) short communications of particularly significant and timely information. Manuscripts will be evaluated by two reviewers.

Articles can be submitted electronically to *invivo@mec.cuny.edu* or mailed as a printed copy (preferably with a diskette that contains the file) to the Editorial Board at Medgar Evers College. All submissions should be formatted double spaced with 1 inch margins. The title of the article, the full names of each author, their academic affiliations and addresses, and the name of the person to whom correspondence should be sent must be given. As a rule, full length articles should include a brief abstract and be divided into the following sections: introduction, materials and methods, results, discussion, acknowledgments and references. Reviews and short communications can be arranged differently. References should be identified in the text by using numerical superscripts in consecutive order. In the reference section, references should be arranged in the order that they appeared in the text using the following format: last name, initials., year of publication. title of article, journal volume number: page numbers. (eg. - [1]Hassan, M. and V. Herbert, 2000. Colon Cancer. *In Vivo* **32:** 3 - 8). For books the order should be last name, initial, year of publication, title of book in italics, publisher and city, and page number referred to. (eg. - Prosser, C.L., 1973. *Comparative Animal Physiology*, Saunders Co., Philadelphia, p 59.). Abbreviations and technical jargon should be avoided. Tables and figures should be submitted on separate pages with the desired locations in the text indicated in the margins.

In This Issue:

Seton Hall University was founded in 1856 by Bishop James Roosevelt Bayley, the first bishop of Newark. It is the oldest diocesan university in the United States.

Nestled on 58 acres in the suburban village of South Orange, NJ, the campus is home to eight schools and colleges: the College of Arts and Sciences, the College of Education and Human Services, the College of Nursing, the John C. Whitehead School of Diplomacy and International Relations, the School of Health and Medical Sciences, the Stillman School of Business, and the Immaculate Conception Seminary School of Theology. Seton Hall's eighth school, the School of Law, is located in Newark, New Jersey. The Division of Continuing Education and Professional Studies is located on the South Orange Campus.

Seton Hall University is founded on and defines itself and its academics, student life and community programs on a Christian understanding of the nature of the world and the human person. With a tradition of quality education based on Christian values, the University takes pride in its concern for the intellectual, ethical and spiritual development of its undergraduate and graduate students.

Seton Hall is committed to bringing together people of different races, cultures, religious traditions, lifestyles and ethnic backgrounds into a community that is respectful and supportive. This commitment has helped to establish a truly multicultural community in which all people of good will are welcome. Seton Hall strives to develop the intellectual, social and religious talents of its students so they may live their lives responsibly, generously and successfully.

At the undergraduate level, Seton Hall offers more than 60 majors and concentrations, as well as many minors, certificates, and interdisciplinary and other special programs.

The Department of Biological Sciences offers programs of study leading to the Bachelor of Arts, Bachelor of Science, Master of Science in Biology, Master of Science in Biology with a Business Administration minor, Master of Science in Microbiology, and Doctor of Philosophy in Molecular Bioscience. The Department of Biological Sciences also offers three dual degree programs: Physical Therapy (B.S./D.P.T.), Physician Assistant (B.S./M.S.P.A.), and Athletic Training (B.S./ M.S.A.T.) with the School of Health and Medical Sciences. At the undergraduate level, the Department of Biological Sciences also offers an interdisciplinary minor program in environmental sciences.

Bactericidal Activity of L-carnitine Against *Salmonella enterica S*erovar Typhimurium

by

Mouhamed Halwani, John M. Jackson, Alexander Vasilakos and Adam J. Houlihan*

Department of Biological Sciences
Wagner College, Staten Island, NY
***current address: Department of Biology**
Randolph College, Lynchburg, VA

Abstract

The antimicrobial activity of L-carnitine against *Salmonella enterica* serovar Typhimurium was evaluated using minimum inhibitory concentration (MIC) and kill curve assays. The MIC for this quaternary ammonium compound was determined to be 3.125 mg/mL. Kill curve assays were conducted by exposing 10^9 cfu/mL of *S. enterica* Typhimurium to L-carnitine at MIC, double MIC (6.25 mg/mL), and half MIC (1.525 mg/mL) and monitoring viable cell count over time. Results revealed a dose-dependent relationship between L-carnitine concentration and the rate of bactericidal activity. These data indicate that L-carnitine, a food additive that is generally regarded as safe (GRAS), can potentially be employed to reduce *S. enterica* Typhimurium.

Introduction

The proliferation and dissemination of antibiotic-resistant bacteria poses a significant threat to human health. Antimicrobial resistance is a natural consequence of the overuse of antimicrobial agents in medicine, agriculture, and the home[1-5]. The declining efficacy of conventional therapeutic antimicrobial agents makes infection control difficult and expensive[6,7]. Therefore, new compounds must be assessed for their antimicrobial properties and potential utility.

Carnitine is a quaternary ammonium compound biosynthesized from the amino acids lysine and methionine[8]. Like amino acids, the L isomer of carnitine is more relevant in biological systems. It was originally found to be a growth factor for mealworms and was categorized as vitamin BT[9]. Today, L-carnitine is marketed as a nutritional supplement and is a common component in energy drinks. During beta-oxidation, L-carnitine is utilized by eukaryotic cells for the transport of fatty acids from the cytosol into the mitochondria via the carnitine shuttle[10-12]. In addition, carnitine has osmoprotectant properties by acting as a compatible solute in bacteria[13]. L-carnitine also plays a role in sperm motility and energy production and helps provide a pathogen free environment in the male urogenital tract[14].

Quaternary ammonium compounds are defined as molecules that include a cationic nitrogen atom covalently bound to four alkyl or aryl functional groups. Quaternary ammonium compounds are potent antimicrobials; they are colorless, unscented, flavorless, stable, easily diluted and non-toxic to tissue[15]. These compounds disrupt cell membranes and denature proteins and are effective against vegetative bacterial cells, protozoa, fungi, and enveloped viruses[15]. They are far less effective against spores and non-enveloped viruses[15].

Quaternary ammonium compounds cause a dissociation of membrane phosphlipid bilayers, directly affecting membrane permeability and resulting in leakage of cellular contents[16]. In addition, many enzymes involved with respiratory and metabolic cellular processes are denatured by quaternary ammonium compounds. Cationic quaternary ammonium compounds disrupt critical intermolecular protein interactions causing loss of tertiary structure.

Despite the routine use of some quaternary ammonium compounds as disinfectants, relatively little information is known about L-carnitine's antimicrobial properties. One report noted that L-carnitine has antimicrobial activity against an array of Gram-positive and Gram-negative bacteria[17]. Another study found that a broad variety of modified L-carnitine esters had antimicrobial activity against the Gram-positive species *Staphylococcus aureus* and *Enterococcus faecalis*, and the Gram-negative species *Escherichia coli* and *Proteus vulgaris*[18]. In another study, acetyl-L-carnitine (ALC), a derivative of L-carnitine, was administered to patients with active pulmonary tuberculosis[19]. The study reported no

decline in pulmonary tubercles over a thirty day period when compared to a placebo group. *Salmonella*, a bacterial genus within family Enterobacteriaceae, is comprised of Gram-negative, rod-shaped, motile, facultative anaerobes that produce H_2S and do not ferment lactose[20]. Salmonellae live in the intestinal tracts of warm and cold blooded animals. Some species, including *Salmonella enterica* serovar Typhimurium (*Salmonella* Typhimurium), cause an acute gastrointestinal illness called salmonellosis. In recent years, problems connected to *Salmonella* have amplified considerably, both in incidence and severity of disease. Moreover, there has been an increased rate of antibiotic-resistant *Salmonella* infections in the United States[2], including outbreaks of multidrug resistant (MDR) *Salmonella* Typhimurium[21]. In this study, we focus on the antibacterial properties of L-carnitine against *Salmonella* Typhimurium.

Materials and Methods

Culture conditions: *Salmonella* Typhimurium cultures were maintained in autoclave-sterilized nutrient broth (Difco) at 37°C with agitation (sub-cultured every 24 hours). Overnight cultures (37°C, 24 hours, with agitation) were used as inocula for the following experiments.

L-Carnitine stock solutions: Stock solutions containing 100 mg/mL (10% [w/v]) L-carnitine were prepared by adding 5 g of L-carnitine to 45 ml of de-ionized water. L-Carnitine was put into solution by gentle warming on a stir plate. The solution was sterilized by vacuum filtration through a 0.22 μm pore size membrane filter (Millipore).

Minimum inhibitory concentration (MIC): Inocula were prepared by adding an overnight culture of *Salmonella* Typhimurium (37°C, 24 hrs, with agitation) to fresh nutrient broth to a final cell density of 10^4 cfu/mL. One hundred μL of inoculated nutrient broth was added to the wells of a 96-well plate (Thermo Scientific). Two-fold serial dilutions of L-carnitine were made by adding 100 μL of the sterile 100 mg/mL aqueous L-carnitine solution to the first well of each inoculated row on the 96 -well plate (final concentration = 50 mg/mL) and serially transferring 100 μL down the 14 cell row. The final dilution contained 0.0061 mg/mL of L-carnitine. To discount osmotic inhibition by L-carnitine at higher concentrations, MIC experiments were repeated using the amino acid glycine as a control. A negative control containing only inoculated nutrient broth was also performed. The 96-well plates were incubated at 37°C for 24 hrs and growth was assessed via optical density measurements using a microplate reader (595 nm wavelength) (Bio Rad).

Bactericidal curves: A washed cell inoculum was created by adding an overnight culture of *Salmonella* Typhimurium (37°C, 24 hrs, with agitation) to 250 mL of fresh nutrient broth and the culture was incubated at 37° C for 150 minutes with agitation. Ten mL of the culture was added to sterile 15-mL conical centrifuge tubes (Falcon) and the tubes were centrifuged at 4,100 rpm for 10 minutes at 4°C. Cell pellets were washed with 10 mL of sterile phosphate buffered saline (PBS), re-centrifuged (4,100 rpm, 4°C, 10 minutes) and re-suspended in PBS to a final cell density of 10^9 cfu/mL. Sterile aqueous L-carnitine was added to the PBS cell suspensions at 1.625 mg/mL (half the determined MIC for L-carnitine against *Salmonella* Typhimurium [see results]), 3.125 mg/mL (MIC), and 6.25 mg/mL (double the MIC). A negative control containing *Salmonella* Typhimurium with no L-carnitine was also prepared. Tubes were incubated at 37°C with agitation and optical density (595 nm wavelength) and viable cell counts were determined every 30 minutes for 120 minutes. In addition, the optical density and viable cell count was determined after 18 hours at 37°C with agitation.

Statistical analyses: All experimental determinations were performed in triplicate. If the coefficient of variation was < 10% and the difference among treatment means was large, statistics were not reported.

Results

Minimum inhibitory concentration (MIC): The MIC for L-carnitine against *Salmonella* Typhimurium was determined to be 3.125 mg/mL. The MIC was also determined using glycine as a control to rule out osmotic inhibition as the mechanism of action for L-carnitine inhibition. The MIC for glycine was determined to be 25 mg/mL. Negative controls showed no inhibition at any point.

Bactericidal curves: All L-carnitine treatments displayed an immediate bactericidal effect when added to *Salmonella* Typhimurium cells in PBS (Figure 1). Indeed, cell densities dropped approximately 10-fold within 5 minutes for all three concentrations tested. At the MIC (3.125 mg/mL) and half the MIC (1.525 mg/mL), cell density dropped approximately two orders of magnitude within 60 minutes and remained steady. At double the MIC (6.25 mg/mL) there was a 1,000,000-fold reduction in viable cell count after 30 minutes, with cell densities dropping from 10^9 cfu/mL to 451 cfu/mL. Viable cell count did not decline appreciably between 30 and 120 minutes.

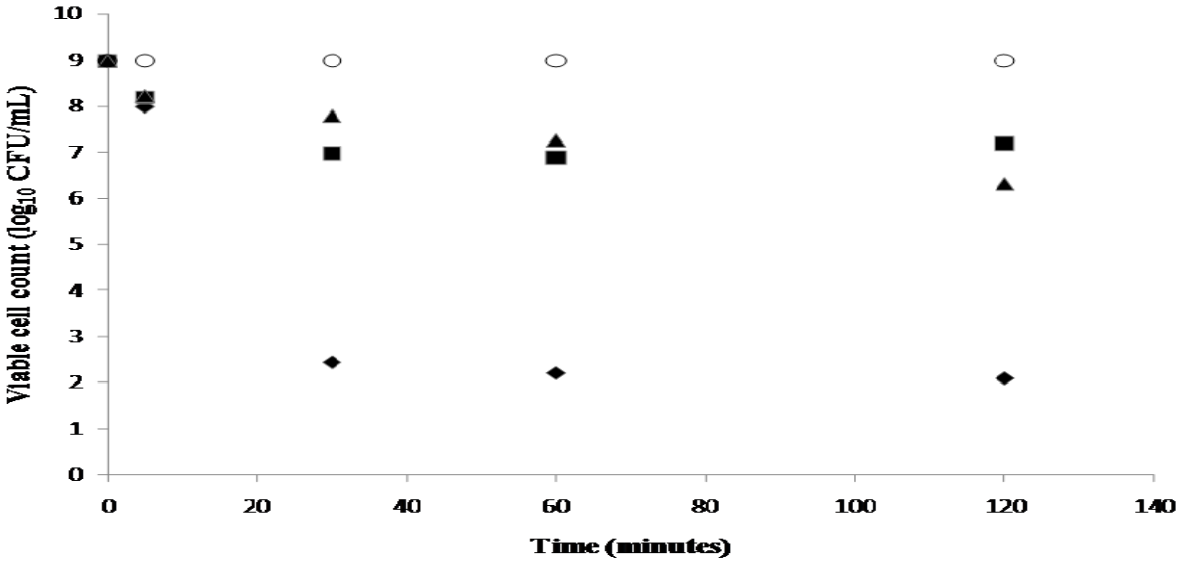

Fig. 1. Kill curves for 0 mg/mL (○), 1.625 mg/mL (▲), 3.125 mg/mL (■) and 6.25 mg/mL (♦) of L-carnitine against *Salmonella* Typhimurium. All experiments were conducted in triplicate and the coefficient of variation for each treatment never exceeded 5%.

Discussion

This work demonstrates that L-carnitine has antimicrobial effects against *Salmonella* Typhimurium. The MIC for L-carnitine against *Salmonella* Typhimurium was determined to be 3.125 mg/mL. To discount osmotic effects, an MIC was also determined using the simple amino acid glycine. The MIC for glycine against *Salmonella* Typhimurium was found to be 25 mg/mL (2.5% [w/v]), which is almost an order of magnitude higher than the MIC observed for L-carnitine. This suggests that inhibition of *Salmonella* Typhimurium by L-carnitine is not simply a result of hypertonicity.

A kill curve demonstrated the bactericidal effect of L-carnitine against 10^9 cfu/mL of *Salmonella* Typhimurium (Figure 1). The results clearly show that 6.25 mg/mL (double the MIC) had the greatest bactericidal effect over the course of the 120 minute incubation. Treatment with the MIC concentration (3.125 mg/mL) and half the MIC (1.525 mg/mL) also displayed inhibitory properties, but caused a viable cell reduction that was 5 orders of magnitude less lethal than double MIC. These results show that L-carnitine inhibits *Salmonella* Typhimurium in a dose-dependent manner.

The chemical structure of L-carnitine differs somewhat from other quaternary ammonium compounds. Unlike most quaternary ammonium compounds, including those that are currently employed as disinfectants, L-carnitine contains functional groups that increase its overall hydrophilicity (Figure 2). Despite slight differences in structure, it can be supposed that L-carnitine's antimicrobial properties result from the same general mechanism of action as related compounds (i.e. disruption of membrane and protein structures).

There is an urgent need for novel antimicrobial agents. The data presented in this paper strongly suggests that L-carnitine has potential as an effective antimicrobial against *Salmonella* Typhimurium. Because L-carnitine is already used as a food additive, it could conceivably be utilized to improve food safety.

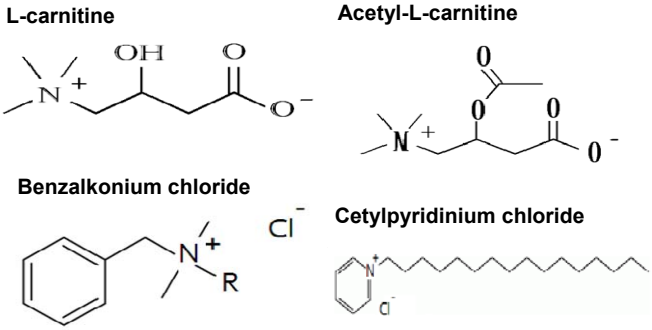

Fig. 2. Structures of L-carnitine, acetyl-L-carnitine and the commercial disinfectants/antiseptics benzalkonium chloride and cetylparadinium chloride. The R of benzalkonium chloride represents an 8, 10, 12, 14, 16 or 18 carbon alkyl group.

To our knowledge, this is the first study that has systematically assessed the bactericidal activity of L-carnitine against the gastrointestinal pathogen *Salmonella* Typhimurium. Future research will include determination of the mechanism of inhibition, as well as synergistic effects with other antimicrobial compounds and environmental conditions.

Acknowledgments

We would like to extend our appreciation to Dr. Roy Mosher and Dr. Kathleen Bobbitt of the Department of Biological Sciences at Wagner College for their input and guidance in the completion of these experiments.

References

[1]Carattoli, A., L. Villa, C. Pezzella, E. Bordi and P. Visca, 2001. Expanding drug resistance through integron acquisition by IncFI plasmids of *Salmonella enterica* Typhimurium. Emerging Infectious Diseases **7:** 444–447.

[2]Besser, T.E., M. Goldoft, L.C. Pritchett, R. Khakhria, D.D. Hancock, D.H. Rice, J.M. Gay, W. Johnson and C.C. Gay, 2000. Multi-resistant *Salmonella* Typhimurium DT104 infections of humans and domestic animals in the Pacific Northwest of the United States. Epidemiology and Infection **124(2):** 193–200.

[3]Bloomfield, S.F., 2002. Significance of biocide usage and antimicrobial resistance in domiciliary environments. Journal of Applied Microbiology **92:** 144S–157S.

[4]McEwen, S.A. and P.J. Fedorka-Cray, 2002. Antimicrobial use and resistance in animals. Clinical Infectous Disease **34:** S93–S106.

[5]Wise, R. and E.J. Soulsby, 2002. Antibiotic resistance - an evolving problem. The Veterinary Record **151:** 371–372.

[6]Moellering, R.C., 1998. Antibiotic resistance: lessons for the future. Clinical Infectious Disease **27:** S135–140.

[7]Vidaver, A.K., 2002. Uses of antimicrobials in plant agriculture. Clinical Infectious Disease **34:** S107–110.

[8]Rebouche, C.J., 1991. Ascorbic acid and carnitine biosynthesis. The American Journal of Clinical Nutrition **54:** 1147S.

[9]Carter, H.E., P.K. Bhattacharyya, K.R. Weidman and G. Fraenkel, 1952. Chemical studies on vitamin BT isolation and characterization as carnitine. Archives of Biochemistry and Biophysics **38:** 405-416.

[10]Kelly, G.S., 1998. L-Carnitine: therapeutic applications of a conditionally-essential amino acid. Alternative Medicine Review **3(5):** 345-360.

[11]Yano, H., E. Oyanagi, Y. Kato, Y. Samejima, J. Sasaki and K. Utsumi, 2010. L-carnitine is essential to beta-oxidation of quarried fatty acid from mitochondrial membrane by PLA(2). Molecular and Cellular Biochemistry **342(1-2):** 95-100.

[12]Steiber, A., J. Kerner and C. Hoppel, 2004. Carnitine: a nutritional, biosynthetic, and functional perspective. Molecular Aspects of Medicine **25(5-6):** 455–73.

[13]Maras, M., Y. Akman, E. Yula and T. Gökmen, 2010. The osmoprotective effect of L-carnitine factor on the bacterial growth of *Streptococcus agalactiae* and *Escherichia coli*. African Journal of Microbiology Research **4(16):** 1708-1712.

[14]Lenzi, A., F. Lombardo, P. Sgro, P. Salacone, L. Caponecchia, F. Dondero and L. Gandini, 2003. Use of carnitine therapy in selected cases of male factor infertility: a double-blind crossover trial. Fertility and Sterility **79(2):** 292-300.

[15]Block, S.S., 2000. *Disinfection, Sterilization, and Preservation (5th edition)*, Lippincott, Williams & Wilkins, p 481.

[16]Thorsteinsson, T., T. Loftsson and M. Masson, July 2003. Soft Antibacterial agents. Current Medicinal Chemistry **10(13):** 1129-1136.

[17]Olgun, A., O. Kisa, S.T. Yildiran, S. Tezcan, S. Akman and M.K. Erbil, 2004. Antimicrobial efficacy of L-carnitine. Annals of Microbiology **54(1):** 95-101.

[18]Calvani, M., L. Critelli, G. Gallo, F. Giorgi, G. Gramiccioli, M. Santaniello, N. Scafetta, M.O. Tinti and F. De Angelis, 1998. L-carnitine esters as "soft", broad-spectrum antimicrobial amphiphiles. Journal of Medicinal Chemistry **41(13):** 2227-2233.

[19]Jirillo, E., M. Altamura, I. Munno, N.M. Pellegrino, R. Sabato, S. Di Fabio and C. De Simone, 1991. Effects of acetyl-L-carnitine oral administration on lymphocyte antibacterial activity and TNF-alpha levels in patients with active pulmonary tuberculosis. A randomized double blind versus placebo study. Immunopharmacology and Immunotoxicology **13(1-2):** 135-146.

[20]Hong, Y., T. Liu, M.D. Lee, C.L. Hofacre, M. Maier, D.G. White, S. Ayers, L. Wang, R. Berghaus and J.A. Maurer, 2009. A rapid screen of broth enrichments for *Salmonella enterica* serovars Enteritidis, Hadar, Heidelberg, and Typhimurium by using an allelotyping multiplex PCR that targets O- and H-antigen alleles. Journal of Food Protection **72(10):** 2198-2201.

[21]Glynn, M.K., C. Bopp, W. Dewitt, P. Dabney, M. Mokhtar and F.J. Angulo, 1998. Emergence of multidrug-resistant *Salmonella enterica* Serotype Typhimurium DT104 infections in the United States. New England Journal of Medicine **338:** 1333-1339.

The Ability of PAS, Acetylsalicylic Acid and Calcium Disodium EDTA to Protect Against the Toxic Effects of Manganese on Mitochondrial Respiration in Gill of *Crassostrea virginica*

by

Sherine Crawford, Kiyya Davis, Claudette Saddler, Jevaun Joseph[1], Edward J. Catapane and Margaret A. Carroll

Department of Biology, Medgar Evers College, Brooklyn, NY
[1]Department of Biological Sciences, Kingsborough Community College, Brooklyn, NY

Abstract

Manganese (Mn) is an essential metal that at excessive levels in brain causes Manganism, a condition similar to Parkinson's disease. Previously we showed that Mn had a neurotoxic effect on the dopaminergic, but not serotonergic, innervation of the lateral ciliated cells in the gill of the Eastern Oyster, *Crassostrea virginica*. While the mechanism of action of Mn toxicity is not completely understood, studies suggest that Mn toxicity may involve mitochondrial damage and resulting neural dysfunction in the brain's dopaminergic system. In this study we utilized micro-batch chambers and oxygen probes to measure oyster gill mitochondrial respiration in the presence of Mn and potential Mn blockers. The addition of Mn to respiring mitochondria caused a dose dependent decrease in mitochondrial O_2 consumption. Pretreating mitochondria with calcium disodium EDTA (caEDTA), p aminosalicylic acid (PAS) or acetylsalicylic acid (ASA) before Mn additions, provided full protection against the toxic effects of Mn. While mitochondrial pretreatment with any of the 3 drugs effectively blocked Mn toxicity, none of the drugs tested was able to reverse the decrease in mitochondrial O_2 consumption seen in Mn treated mitochondria. The study found that high levels of Mn had a toxic effect on gill mitochondrial O_2 consumption and that this effect could be blocked by the drugs caEDTA, PAS and ASA. *C. virginica* continues to be a good model with which to investigate the mechanism that underlies manganese neurotoxcity and in the pharmacological study of drugs to treat or prevent Manganism.

Introduction

Manganese is an element present in all animal tissues and required as an enzyme cofactor or activator for numerous reactions of metabolism[1]. While essential in trace amounts, excessive manganese exposure can result in toxic accumulations in human brain tissue and resulting extrapyramidal symptoms similar to those seen in patients with Idiopathic Parkinson s disease[2-5]. This Parkinson-like neurological condition first described in 1837 in two manganese ore-crushing mill workers[6] has been referred to as Manganism[7-10]. Inhalation of manganese from the atmosphere is believed to be the primary cause of manganese toxicity[11]. In addition to mining and manganese ore processing, high levels of airborne manganese are possible in a number of other occupational settings, including welding, dry battery manufacture, and use of certain organochemical fungicides like Maneb[12-16]. More recently, questions are being asked about the safety of ambient manganese in the general population and there is a growing concern that chronic low-level occupational or increased environmental exposure to manganese may be a contributing factor in a variety of neurological conditions including the high numbers of people diagnosed with Parkinson's disease in the United States and elsewhere[4,17-22].

Although manganese toxicity has been recognized for some time the primary mechanism underlying its neurotoxic effects remains elusive. Clinically, Manganism resembles Idiopathic Parkinson's disease, a dopaminergic cell disorder. Symptoms common to both disorders include gait imbalance, rigidity, tremors and bradykinesia[7,23-25], suggesting a similar etiology of neuronal damage in the substantia nigra with a resulting deficiency of the neurotransmitter dopamine for the striatum. However, compared to Parkinson's, there are some differentiating features seen with Manganism including symmetry of effects, more prominent dystonia, a characteristic cock walk, an intention rather than resting tremor, earlier behavioral and cognitive dysfunction, difficulty turning, and a poor response to Levodopa[2,16,21,26-30] suggesting different or more extensive damage in the basal ganglia or to the dopaminergic system. Human and animal studies have shown

that toxic exposure to manganese results in metal accumulations in various areas of the basal ganglia and dysfunction of cells of both the striatum and the globus pallidus[31-36]. Manganese selectively targets dopaminergic neurons in the human basal ganglia[16,35] and decreases dopamine levels in the striatum[23,37-40]. Considering the clinical similarities between Manganism and Parkinson's Disease, and the fact that manganese accumulates in brain regions rich in dopaminergic neurons, it has long been suggested that manganese neurotoxicity involves a disruption in dopaminergic neurotransmission[41-43].

While the cellular and molecular mechanism of manganese toxicity remains unclear, evidence suggest that exposure to manganese or manganese containing compounds induces oxidative stress-mediated dopaminergic cell death[44-46] which is in agreement with current theories on oxidative stress as a mediator of neuronal death in Parkinson's disease and other neurodegenerative diseases[47-51]. When in excess, manganese has been shown to raise levels of reactive oxygen species[52-55].

Dopamine, serotonin and other biogenic amines are present in the nervous tissue and gill of the Eastern Oyster, *Crassostrea. virginica*[56]. The nervous system innervation of the lateral ciliated cells in the gill of *C. virginica* is composed of a cilio-excitatory serotonergic system and a cilio-inhibitory dopaminergic system[57]. *C. virginica* are sedimentary animals known to accumulate metals in their tissues from waters where they reside. The Environmental Protection Agency (EPA) lists them as test animals for studying marine pollutants. Since *C. virginica* provides a relatively simple system with a serotonergic-dopaminergic innervation component that directs an observable and measurable physiological response, this system can be useful in investigating the mechanisms that underlie both manganese neurotoxicity and other dopaminergic cell disorders.

Previously we showed that manganese had a neurotoxic effect on the dopaminergic, but not serotonergic, innervation of the lateral ciliated cells in the gill of *C. virginica* and that *C. virginica* treated with manganese also had lowered endogenous dopamine levels in the gill, cerebral ganglia and visceral ganglia[56,58,59]. We showed that the loss of endogenous dopamine could be prevented by co-treating the animals with PAS (p-aminosalycilic acid)[56], a drug that has recently been shown as a successful therapeutic agent for Manganism[60], but the mechanism of PAS action is unclear. In other experiments, adding PAS or the metal chelator EDTA to oyster gill blocked the neurotoxic effects of manganese on the dopaminergic innervation of the gill lateral ciliated cells[59].

The present study sought to investigate the effects of manganese on gill mitochondrial respiration. Preliminary work in our lab suggested that manganese reduced gill mitochondrial respiration in *C. virginica*. We also sought to test potential drugs such as EDTA, PAS and ASA (acetylsalicylic acid) to determine if they would have a blocking effect on manganese toxicity on mitochondrial respiration.

Materials and Methods

Adult oysters *C. virginica* of approximately 80 mm shell length were obtained from Frank M. Flower & Sons oyster farm in Oyster Bay, NY. Animals were transported on ice to Medgar Evers College within 2 hours of collection and placed in recirculating aquaria with artificial seawater (Instant Ocean , Aquarium Systems Inc., Mentor, OH) at 16-18°C, specific gravity of 1.024 ± 0.001 and pH of 7.8 ± 0.2. Animals were used in the experiments within one week of arriving in the lab. Each animal was tested for health prior to experimentation by the resistance it offered to being opened. Animals that fully closed in response to tactile stimulation and required at least moderate hand pressure to being opened were used for the experiments.

PAS and ASA were obtained from Sigma-Aldrich, St. Louis, MO. All other chemicals were obtained from Fisher Scientific, Pittsburgh, PA. Mitochondria were isolated from oyster gills. The isolation buffer consisted of 540 mM glycine, 250 mM sucrose, 2 mM EDTA, 20 mM Tris-HCl, pH 7.4, and 0.2% bovine serum albumin (BSA, essentially fatty acid free). For each experimental trial, the gills from one animal were removed, blotted dry, weighed and placed in 10 ml of ice cold isolation medium. The tissue was homogenized with five passes of a Potter-Elvehjem homogenizer equipped with a loosely fitting Teflon pestle. The homogenate was centrifuged at 4°C for 10 min at 600 X g in a Sorval RC5B centrifuge to remove the nuclear pellet. The supernatant was centrifuged at 4°C for 20 min at 10,000 X g to pellet the mitochondria. The resulting mitochondrial pellet was washed twice with ice-cold EDTA-free isolation buffer to minimize binding by the chelator and resuspended

in 2 ml of ice cold EDTA-free isolation buffer. Oxygen uptake by mitochondria was measured in a Micro-Biological Oxygen Monitor with a 600 µl micro -batch chamber (YSI, Yellow Springs, OH). Continuous data acquisition was made using a DI-700 Data Acquisition System (DATAQ Instruments, Inc., Akron, OH). Temperature in the mitochondrial respiration chamber was maintained constant at 20° C using a Haake water circulator. The microbatch chamber was filled with respiratory buffer (0.25 succinate, 0.05 mM ADP, 5 mM $MgCl_2$, 10 mM K_2HPO_4, 540 mM glycine, 250 mM sucrose, 20 mM Tris-HCl, pH 7.4, 0.2% BSA) and allowed to equilibrate. After the baseline was stable for 5 minutes an aliquot (100 µl) of isolation medium containing mitochondria was added to the chamber and basal mitochondrial respiratory rates (state 3) were measured. After 10 minutes, aliquots (20 µl) of Mn, caEDTA, PAS or ASA were added to the chamber to determine their effects on respiratory rates. N ranged from 5-22 and statistical analyses were determined by ANOVA with Tukey post test.

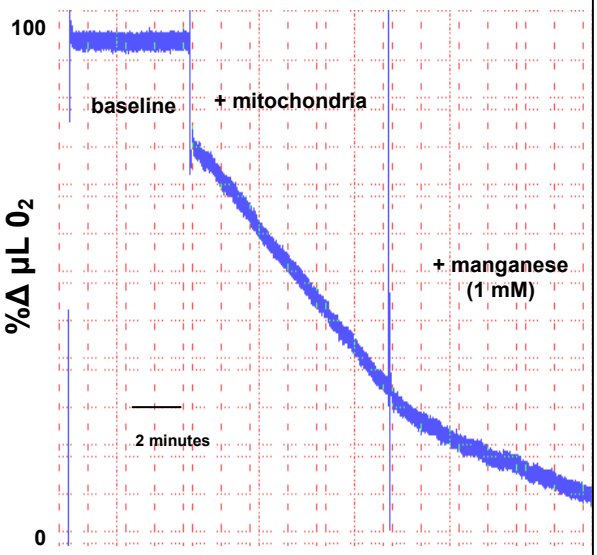

Fig. 1. Mitochondrial oxygen consumption using a Yellowsprings 5300 Biological Oxygen Monitor. Baseline = the equalization before addition of mitochondria. 100% saturation = 6.51 µL O_2 / ml at 20°C.

Results

The basal oxygen utilization rate for isolated mitochondria from 22 gill samples was 6.18 ± 0.65 µLO_2/hr/gm. Figure 1 illustrates results of a typical run. The baseline is the equilibration period without mitochondria. The steady downward slope after mitochondrial addition demonstrates oxygen consumption of respiring (state 3) mitochondria. Adding manganese (1 mM) decreased the magnitude of the slope as a result of a decreased oxygen consumption. Figure 2 shows that manganese causes a dose dependant decrease in mitochondrial O_2 consumption. Adding up to 1 mM manganese caused a 60% drop in mitochondrial oxygen consumption. Higher doses resulted in greater decreases.

Figures 3a, 4a and 5a show the effects of adding 1 mM of PAS, ASA and caEDTA , respectively, to the microbatch chambers prior to 1 mM manganese additions. None of the drugs had significant effects of their own on basal mitochondrial oxygen consumption. However, each drug fully blocked the toxic effects of manganese on mitochondrial oxygen consumption under the conditions of the experiments. Each of the figures show that when the drug was added prior to manganese there was no significance difference between basal and manganese treated mitochondria.

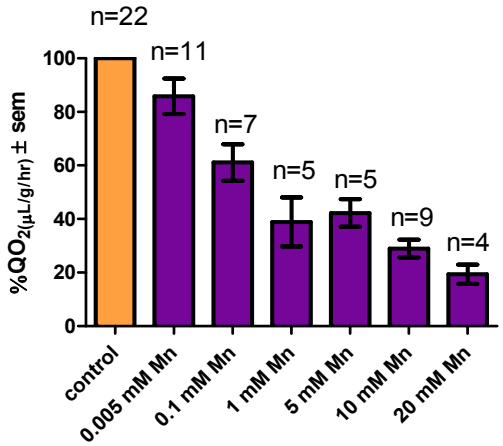

Fig. 2. Mitochondrial oxygen consumption in the presence of manganese compared to control. Statistical analysis was determined by ANOVA with Tukey post test, $p<0.01$ for each treatment compared to control.

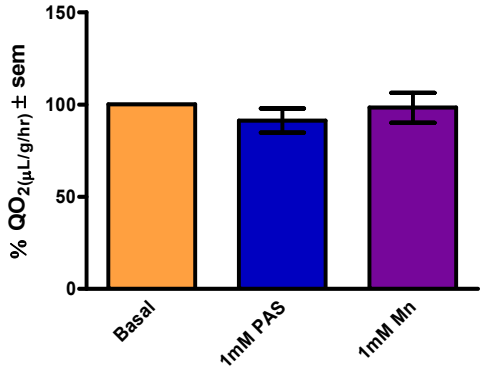

Fig. 3a. Effects of PAS on mitochondrial oxygen consumption when applied before manganese. Statistical analysis was determined by ANOVA with Tukey post test, n = 6.

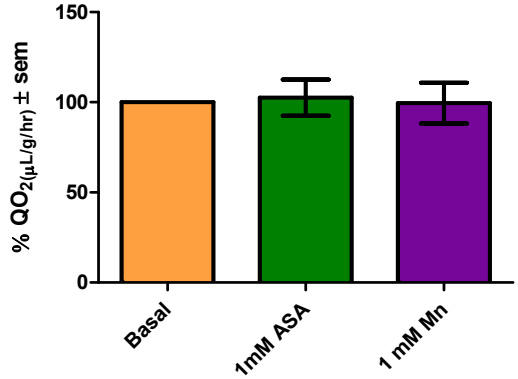

Fig. 4a. Effects of ASA on mitochondrial oxygen consumption when applied prior to manganese. Statistical analysis was determined by ANOVA with Tukey post test, n = 5.

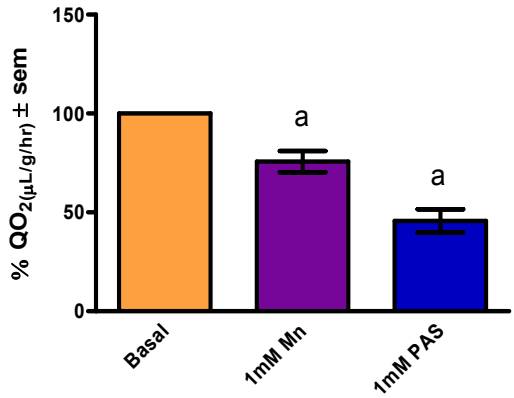

Fig. 3b. Effects of PAS on mitochondrial oxygen consumption when applied after manganese. Statistical analysis was determined by ANOVA with Tukey post test, n = 10, [a]p<0.01 compared to basal.

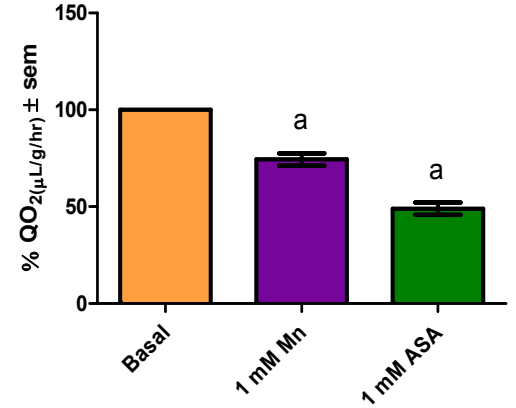

Fig. 4b. Effects of ASA on mitochondrial oxygen consumption when applied after manganese. Statistical analysis was determined by ANOVA with Tukey post test, n = 14, [a]p<0.01 compared to basal.

Figures 3b, 4b and 5b show the effects of adding 1 mM of PAS, ASA and caEDTA, respectively, to the microbatch chambers after 1 mM manganese additions. PAS (Fig. 3b) and ASA (Fig. 4b) were ineffective in reversing the toxic effects of manganese when added after manganese. The manganese-induced drop in mitochondrial oxygen consumption continued to decrease despite the additions of PAS or ASA. When caEDTA was added after manganese (Fig. 5b), it did not reverse the toxic effects of manganese, but in contrast to PAS and ASA, caEDTA did prevent any further drop in oxygen consumption.

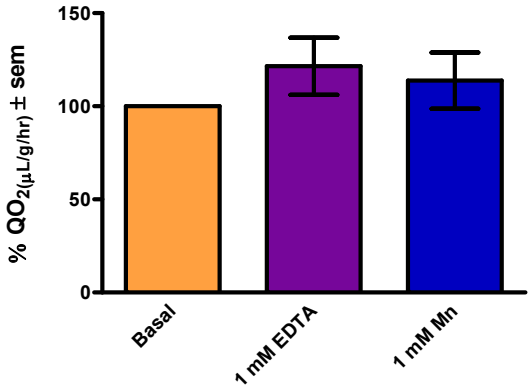

Fig. 5a. Effects of EDTA on mitochondrial oxygen consumption when applied after manganese. Statistical analysis was determined by ANOVA with Tukey post test, n = 7.

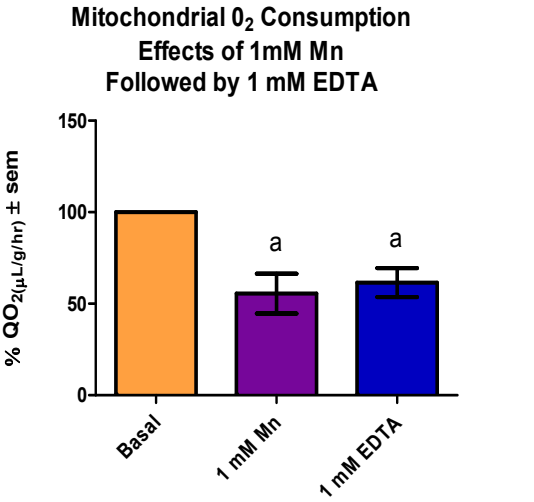

Fig. 5b. Effects of EDTA on mitochondrial oxygen consumption when applied after manganese. Statistical analysis was determined by ANOVA with Tukey post test, n = 6, [a]p<0.01 compared to basal.

Discussion

Manganese is a trace element in animal systems required for normal carbohydrate, lipid, amino acid and protein metabolism, as well as a required cofactor for various antioxidant enzymes such as mitochondrial superoxide dismutase[1,61], however high levels can cause the neurodegenerative disorder known as Manganism[2-5]. While the cellular and molecular mechanism of manganese toxicity remains unclear, several lines of evidence suggest that exposure to manganese or manganese-containing compounds induces oxidative stress-mediated dopaminergic cell death[46,62,63] which is in agreement with current theories on oxidative stress as a mediator of neuronal death in Parkinson's disease and other neurodegenerative diseases[48,64-67].

Dopaminergic neurons and dopamine-rich areas of the brain are particularly vulnerable to oxidative stress, because the enzymatic and non-enzymatic metabolism of dopamine can generate reactive oxygen species and various neurotoxic catecholamine metabolites such as 6-hydroxydopamine[66,68-71]. Oxidative stress is also suspected of being a factor in 1-methyl-4-phenyl-1,2,3,6-tetrahyrdopyridine (MPTP)-induced Parkinson's disease because transgenic mice who overexpressed copper/zinc superoxide dismutase were protected from the dopaminergic neuronal degeneration caused by MPTP exposure[73].

Previous work of our lab showed that manganese reduced dopamine levels in the ganglia and innervated tissues of *C. virginica* and disrupted the dopamine innervation of the lateral gill cilia[58,59]. We now show that manganese has a deleterious effect on O_2 utilization in gill mitochondria of *C. virginica*.

This study also provides evidence of the protective actions of three drugs, PAS, ASA and caEDTA, as potential manganese blockers with respect to mitochondrial respiration. EDTA is a well known metal chelator and caEDTA has been used in chelation therapy of various metal poisoning conditions, including mercury and lead[73]. PAS and ASA are structurally related chemicals with anti-inflammatory properties. Jiang *et al.* speculated that the mechanism of action of PAS in alleviating Manganism may be due to its anti-inflammatory ability or its ability to chelate manganese[60]. ASA is known to chelate iron[74], and may have manganese chelating abilities as well. As seen in this study, both PAS and ASA effectively blocked the toxic effects on manganese when applied prior to manganese, but neither

prevented further toxic effects of manganese on mitochondrial oxygen consumption when applied after manganese. Only caEDTA was able to prevent further toxicity when applied after manganese.

The present study demonstrates that *C. virginica* can be used to investigate the mechanism that underlies manganese neurotoxcity, and may also serve as a model in the pharmacological study of drugs to treat or prevent Manganism and perhaps other dopaminergic cell disorders. The toxic effects of manganese on oyster mitochondria and the protection by PAS, ASA and caEDTA may be of significance in understanding the mechanism of actions of manganese in causing Manganism and possible therapeutic treatments.

Acknowledgments

We wish to thank Frank M. Flower and Sons Oyster Farm, Oyster Bay, NY for supplying oysters. This work was supported in part by grants 2R25GM06003 of the Bridge Program of NIGMS, 0516041071 of NYSDOE and 0622197 of the DUE Program of NSF.

References

[1]Cotzias, G.C., 1958. Manganese in health and disease. Physiol. Rev. **38:** 503-532.

[2]Calne, D.B., N.S. Chu, C.C. Huang, C.S. Lu and W. Olanow, 1994. Manganism and idiopathic parkinsonism: similarities and difference. Neurology **44:** 1583-1586.

[3]Aschner, M., 2000. Manganese in health and disease: from transport to neurotoxicity. In: Massaro, E. (Ed.), *Handbook of Neurotoxicology*. Humana Press, Totowa, NJ, pp.195-209.

[4]Levy, B.S. and W.J. Nassetta, 2003. Neurological effects of manganese in humans: a review. Int. J. Occup. Environ. Health **9:** 153-163.

[5]Dobson, A.W., K.M. Erikson and M. Aschner, 2004. Manganese neurotoxicity. Ann. N.Y. Acad. Sci. **1012:** 115-128.

[6]Couper, J., 1837. On the effects of black oxide of manganese when inhaled into the lungs. Br. Ann. Med. Pharmacol. **1:** 41-42.

[7]Mena, I., O. Marin, S. Fuenzalida and G.C. Cotzias, 1967. Chronic manganese poisoning. Clinical picture and manganese turnover. Neurology **17:** 128-136.

[8]Barbeau, A., 1984. Manganese and extrapyramidal disorders (a critical review and tribute to Dr. George C Cotzias). Neurotoxicology **5:** 13-35.

[9]Donaldson, J., 1987. The physiopathologic significance of manganese in brain: its relation to schizophrenia and neurodegenerative disorders. Neurotoxicology **8:** 451-462.

[10]Gorell, J.M., B.A. Rybicki, C. Cole Johnson and E.L. Peterson, 1999. Occupational metal exposures and the risk of Parkinson's disease. Neuroepidemiology **18:** 303-308.

[11]Andersen, M.E., J.M. Gearhart and H.J. Clewell III, 1999. Pharmacokinetic data needs to support risk assessments for inhaled and ingested manganese. Neurotoxicology **20:** 161-171.

[12]National Academy of Sciences, 1973. Medical and biological effects of environmental pollutants: manganese. National Academy Press, Wash. DC, pp. 1-101.

[13]Meco, G., V. Bonfanti, N. Vanacore and E. Fabrizio, 1994. Parkinsonism after chronic exposure to the fungicide maneb (manganese ethylene-bis-dithiocarbamate. Scand. J. Work Environ. Health **20:** 301-305.

[14]Reidy, T.J., R.M. Bowler, S.S. Rauch and G.I. Pedroza, 1992. Pesticide exposure and neuropsychological impairment in migrant farm workers. Arch. Clin. Neuropsychol. **17:** 85-95.

[15]Iregren, A., 1999. Manganese neurotoxicity in industrial exposures: proof of effects, critical exposure level, and sensitive tests. Neurotoxicology **20:** 315-324.

[16]Olanow, C.W., 2004. Manganese-induced parkinsonism and Parkinson's disease. Ann. N.Y. Acad. Sci. **1012:** 209-223.

[17]Mergler, D., 1999. Neurotoxic effects of low level exposure to manganese in human populations. Environmental Research **80(2):** 99-102.

[18]Lucchini, R., P. Apostoli, C. Perrone, D. Placidi, E. Albini and P. Migliorati, 1999. Long-term exposure to “low levels” of manganese oxides and neurofunctional changes in ferroalloy workers. Neurotoxicity **20:** 287–297.

[19]Davis, J.M., 1999. Inhalation health risks of manganese: an EPA perspective. Neurotoxicology **20:** 511–518.

[20]Kaiser, J., 2003. Manganese: a high octane dispute. Science **300:** 926-928.

[21]Jankovic, J., 2005. Searching for a relationship between manganese and welding and Parkinson's disease. Neurology **64:** 2021-2028.

[22]Dorman, D.C. and B.A. Wong, 2006 Neurotoxicity of inhaled manganese: A reanalysis of human exposure arising from showering. Medical Hypotheses **66(1):** 199-200.

[23]Mena, I., J. Court, S. Fuenzalida, P.S., Papavasiliou and G.S. Cotzias, 1970. Modification of chronic manganese poisoning. N. Engl. J. Med. **282:** 5-10.

[24]Rosenstock, H.A., D.G. Simons and J.S. Meyer, 1971. Chronic Manganism. Neurologic and laboratory studies during treatment with levodopa. JAMA **217:** 1354-1358.

[25]Huang, C.C., N.S. Chu, C.S.Lu, J.D. Wang, J.L. Tsai and J.L. Tzeng, 1989. Chronic manganese intoxication. Arch. Neurol. **46:** 1104-1106.

[26]Barbeau, A., N. Inoué and T. Cloutier, 976. Advances in Neurology **14:** 339-352.

[27]Huang, C.C., C.S. Lu, N.S. Chu, F. Hochberg, D. Lilienfeld and W. Olanow, 1993. Progression after chronic manganese exposure. Neurology **43:** 1479-1483.

[28]Lu, C.S., C.C. Huang, N.S. Chum and D.B. Calne, 1994. Levodopa failure in chronic manganism. Neurology **44:** 1600-1602.

[29]Koller, W.C., K.E. Lyons and W. Truly, 2004. Effect of levodopa treatment for Parkinsonism in welders. A double-blind study. Neurology **62:** 730-733.

[30]Cersosimo, M.G. and W.C. Koller, 2006. The diagnosis of manganese-induced parkinsonism. NeuroToxicology **27:** 340-346.

[31]Eriksson, H., P.G. Gillberg, S.M. Aquilonius, K.G. Hedstriim and E. Heilbronn, 1992. Receptor alteration in manganese intoxicated monkeys. Arch. Toxicol. **66:** 359–364.

[32]Brenneman, K.A., R.C. Cattley, S.F. Ali and D.C.Dorman, 1999. Manganese induced developmental neurotoxicity in the CD rat: is oxidative damage a mechanism of action? Neurotoxicology **20:** 477-488.

[33]Nagatomo, S., F. Umehara, K. Hanada, Y. Nobuhara, S. Takenaga, K. Arimura and M. Osame, 1999. Manganese intoxication during total parenteral nutrition: report of two cases and review of the literature. J. Neurol. Sci. **162:** 102-105.

[34]Newland, M.C., 1999. Animal models of manganese's neurotoxicity. Neurotoxicology. **20:** 415-432.

[35]Pal, P.K., A. Samii and D.B. Calne, 1999. Manganese neurotoxicity: a review of clinical features. Neurotoxicology **20:** 227-238.

[36]Baek, S.Y., M.J. Lee, H.S. Jung, H.J. Kim, C.R. Lee, C. Yoo, J.H. Lee, H. Lee, C.S. Yoon, Y.H. Kim, J. Park, J.W. Kim, B.S, Jeon and Y. Kim, 2003. Effect of manganese exposure on MPTP neurotoxicities. Neurotoxicology **24:** 657-665.

[37]Parenti, M., C. Flauto, E. Parati, A. Vescovi and A. Groppetti, 1986. Manganese neurotoxicity: effects of L-DOPA and pargyline treatments. Brain Res. **367:** 8-13.

[38]Eriksson, H., K. Magiste, L.O. Plantin, F. Fonnum, K. Hedstrtm, G. Theodorsson, E. Norheim, K., Kristensson, E., Stalberg and E. Heilbronn, 1987. Effects of manganese oxide on monkeys as revealed by a combined neurochemical, histological and neurophysiological evaluation. Arch. Toxicol. **61:** 46-52.

[39]Vescovi, A., L. Facheris, A. Zaffaroni, G. Malanca and E.A. Parati, 1991. Dopamine metabolism alterations in a manganese-treated pheochromocytoma cell line (PC12). Toxicology **67:** 129-142.

[40]Sistrunk, S.C., M.K. Ross and N.M. Filipov, 2007. Direct effects of manganese compounds on dopamine and its metabolite DOPAC: an in vivo study. Environ. Toxicol. Pharmacol. **23:** 286-296.

[41]Neff, N.H., R.E. Barrett and E. Costa, E., 1969. Selective depletion of caudate nucleus dopamine and serotonin during chronic manganese dioxide administration to squirrel monkeys. Cellular and Molecular Life Sciences **25(11):** 1140-1141.

[42]Hornykiewicz, O., 1972. Dopamine and extrapyramidal motor function and dysfunction. Res. Publ. Assoc. Res. Nerv. Ment. Dis. **50:** 390-415.

[43]Graham, D.G., 1984. Catecholamine toxicity: a proposal for the molecular pathogenesis of manganese neurotoxicity and Parkinson's disease. Neurotoxicology **5:** 83-95.

[44]HaMai, D. and S.C. Bondy, 2004. Oxidative basis of manganese neurotoxicity, Annals of the New York Academy of Sciences **1012:** 129-141.

[45]Anantharam, V., M. Kitazawa, J. Wagner, S. Kaul, and A.G. Kanthasamy, 2002. Caspase-3 -dependent proteolytic cleavage of protein kinase C delta is essential for oxidative stress-mediated dopaminergic cell death after exposure to methylcyclopentadienyl manganese tricarbonyl. Journal of Neuroscience **22:** 1738 - 1751.

[46]Latchoumycandane, C., V. Anantharam, M. Kitazawa, Y. Yang, A. Kanthasamy and A.G. Kanthasamy, 2005. Protein kinase C delta is a key downstream mediator of manganeseinduced apoptosis in dopaminergic neuronal cells. Journal of Pharmacology and Experimental Therapeutics 313: 46 - 55.

[47]Fahn, S. and G. Cohen, 1992. The oxidant stress hypothesis in Parkinson's disease: evidence supporting it. Annals of Neurology **32:** 804 - 812.

[48]Albers, D.S. and M.F. Beal, 2000. Mitochondrial dysfunction and oxidative stress in aging and neurodegenerative diseases. Journal of Neural Transmission Suppl. **59:** 133 - 154.

[49]Schulz, J.B., J. Lindenau, J. Seyfried and J. Dichgans, 2000. Glutathione, oxidative stress and neurodegeneration. European Journal of Biochemistry **267:** 4904 - 4911.

[50]Dawson, T.M. and V.L. Dawson, 2003. Molecular pathways of neurodegeneration in Parkinson's disease. Science **302:** 819-822.

[51]Emerit, J., M. Edeas and F. Bricaire, 2004. Neurodegenerative diseases and oxidative stress. Biomedicine and Pharmacotherapy **58:** 39 - 46.

[52]Ali, S.F., H.M. Duhart, G.D. Newport, G.W. Lipe and W. Slikker Jr., 1995. Manganese-induced reactive oxygen species: Comparison between Mn^{+2} and Mn^{+3}. Neurodegeneration **4:** 329-334.

[53]Shi, X.L. and N.S. Dalal, 1990. The glutathionyl radical formation in the reaction between manganese and glutathione and its neurotoxic implications. Med-Hypotheses **33(2):** 83 - 87).

[54]Brouillet, E.P.L., U. Shinobu, F. McGarvey, F. Hochberg and M.F. Beal, 1993. Managnese injection into the rat striatum produces excitotoxic lesions by impairing energy metabolism. Experimental Neurology **120:** 89 - 94.

[55]Archibald, F.S. and C. Tyree, 1987. Manganese poisoning and the attack of trivalent manganese upon catecholamines. Archives of Biochemistry and Biophysics **256:** 638 - 650.

[56]King, C., M. Myrthil, M.A., Carroll and E.C. Catapane, 2008. Effects of p-aminosalicylic acid on the neurotoxicity of manganese and levels of dopamine and serotonin in the nervous system and innervated organs of Crassostrea virginica. In Vivo **29(1):** 29–34.

[57]Carroll, M.A. and E.J. Catapane, 2007. The nervous system control of lateral ciliary activity of the gill of the bivalve mollusc, *Crassostrea virginica*. Comp. Biochem. Physiol. A **148:** 445-450.

[58]Martin, K., T. Huggins, C. King, M.A. Carroll and E.J. Catapane, 2008. The neurotoxic effects of manganese on the dopaminergic innervation of the gill of the bivalve mollusc, *Crassostrea virginica*. Comp. Biochem. Physiol. C **148:** 152-159.

[59]Nelson, M., T. Huggins, R. Licorish, M.A. Carroll and E.J. Catapane, 2010. Effects of p-Aminosalicylic acid on the neurotoxicity ofmanganese on the dopaminergic innervation of the cilia of the lateral cells of the gill of the bivalve mollusc, *Crassostrea virginica.* Comparative Biochemistry and Physiology, Part C **151:** 264-270.

[60]Jiang, W., X. Mo, F. Du, X. Fu, X. Zhu, H. Gao, J. Xie, F. Liao, E. Pira and W. Zheng, 2006. Effective treatment of manganese-Induced occupational Parkinsonism with p-aminosalicylic acid: a case of 17-year follow-up study. J. Occup. Environ. Med. **48:** 644-649.

[62]Anantharam, V., M. Kitazawa, J. Wagner, S. Kaul and A.G. Kanthasamy, 2002. Caspase-3-dependent proteolytic cleavage of protein kinase Cdelta is essential for oxidative stress-mediated dopaminergic cell death after exposure to methylcyclopentadienyl manganese tricarbonyl. J Neurosci **22:** 1738–1751.

[63]Stredrick, D.L., A.H. Stokes, T.J. Worst, W.M. Freeman, E.A. Johnson, L.H. Lash, M. Aschner and K.E. Vrana, 2004. Manganese-Induced Cytotoxicity in Dopamine-Producing Cells. NeuroToxicology **25(4):** 543-553.

[64]Fahn S and G. Cohen. 1992. The oxidant stress hypothesis in Parkinson's disease: evidence supporting it. Ann Neuro **132:** 804-812.

[65]Schulz, J.B., Lindenau, J., Seyfried, J., Dichgans, J., 2000. Glutathione, oxidative stress and neurodegeneration. Eur. J. Biochem. **267:** 4904 -4911.

[66]Dawson, T.M. and V.L. Dawson, 2003. Molecular pathways of neurodegeneration in Parkinson's disease. Science **302:** 819-822.

[67]Emerit, J., M. Edeas and F. Bricaire, 2004. Neurodegenerative diseases and oxidative stress. Biomedecine and Pharmacotherapy **58 (1):** 39-46.

[68]Halliwell, B., 1992. Reactive oxygen species and the central nervous system. J. Neurochem. **59:** 1609-1623.

[69]Lotharius, J. and P. Brundin, 2002. Impaired dopamine storage resulting from alpha-synuclein mutations may contribute to the pathogenesis of Parkinson's disease. Hum. Mol. Genet. **11:** 2395–2407.

[70]Cantuti-Castelvetri, I., B. Shukitt-Hale and J.A. Joseph, 2003. Dopamine neurotoxicity: age-dependent behavioral and histological effects. Neurobiology of Aging **24(5):** 697-706.

[71]Dauer, W. and S. Przedborski, 2003. Parkinson's Disease: Mechanisms and Models. Neuron **39 (6):** 889-909.

[72]Przedborski, S., V. Kostic, V. Jackson-Lewis, A.B. Naini, S. Simonetti, S. Fahn, E. Carlson, C.J. Epstein and J.L. Cadet, 1992. Transgenic mice with increased Cu/Zn-superoxide dismutase activity are resistant to N-methyl-4-phenyl-l,2,3,6-tetrahydropyridine-induced neurotoxicity. J Neurosci **12:**1658-1667.

[73]Gervasio A., S. Lamas and J. Hussein, 2006. EDTA chelation therapy meets evidence-based medicine Complementary Therapies in Clinical Practice **12(3):** 213-215.

[74]Schwarz, K.B., B.J. Arey, K. Tolman and S. Mahanty, 1988. Iron chelation as a possible mechanism for aspirin-induced malondialdehyde production by mouse liver microsomes and mitochondria. J Clin Invest **81 (1):** 165-170.

CALL FOR NOMINATIONS

The terms of office for the following positions will be up for reelection to serve on the Year 2011-2012 Executive Board

Vice-President
Treasurer
Recording Secretary
Members-at-Large, 2 positions

The duties of these officers will involve attending all Executive Board meetings in addition to specific duties as described below:

The Vice President will establish and serve as chairperson of the Advisory Council. In the event the President is no longer able to serve, the Vice President will automatically succeed to the presidency for the remainder of the term.

The Treasurer of the Association is responsible for the preparation of an annual fiscal report, processing of dues, preparing regular financial reports for the Executive Board meetings, income tax reports, and other duties usually pertaining to this office.

The Recording Secretary shall record Board Members who are present, absent, or excused from Executive Board meetings and shall distribute the minutes of the Executive Board meetings, the annual business meeting, and any other officially sanctioned meetings as advised by the Executive Board. The Recording Secretary is responsible for Election Committee duties as stated in Article VIII - of these Bylaws.

The Members-at Large shall chair committees (Articulation, Exhibition, etc.) and handle other assignments as directed by the Executive Board.

Normally, each candidate for Vice-President, Recording Secretary and Treasurer should have been a Member-at- Large for at least one term and each candidate for Member-at-Large should have attended at least one Annual Conference.

DEADLINE FOR NOMINATIONS is October 15, 2011

If you are interested in running for office (or wish to nominate anyone else), please send a letter of nomination to:

Dr. Margaret A. Carroll
Biology Department
Medgar Evers College
1638 Bedford Ave
Brooklyn, NY 11225

Metropolitan Association of College and University Biologists

Benjamin Cummings/MACUB 2012 Student Research Grants

Purpose

To provide investigative research support for undergraduate students working under the supervision of faculty who are current members of MACUB. Awards Applications will be evaluated and awards granted based on the scientific merit and overall quality of the proposed research experience.

1. **Four grants of $500 each will be awarded annually (provided by BC).**
2. **Complimentary registration for the annual fall conference of MACUB and membership in MACUB for student research grant awardees (provided by MACUB).**

Eligibility

1. **Only undergraduate students working under the supervision of faculty who is a current member of MACUB may apply.**
2. **Undergraduates who are graduating seniors must plan to complete their research prior to graduation.**
3. **A student is only eligible to receive one award.**

Requirements

1. **Student research grants may be used to support scientific investigation in any field of biology.**
2. **Funding may be used to purchase equipment or supplies required for the proposed project, and/or travel to and from a research location.**
3. **Grant winners are required to present the results of research supported by this award at the MACUB annual fall conference following the year of the award.**
4. **Institutional support is required. This may include research supplies, travel expenses, in-kind matches, and other forms of support.**
5. **All application materials must be submitted on-line at http//www.macub.org by February 28, 2012 and all applicants will receive notification of award status by March 15, 2012.**

You are invited to participate in the 44th Annual Fall MACUB Conference

Register on line at the MACUB Website (www.macub.org) or by mail by completing the registration form on page 18

Member Presentations

Members wishing to make a 2011 Member Presentation (20 min) to discuss the results of research or share ideas, should contact

Dr. Carolyn S. Bentivegna
at Caroyn.Bentivegna@shu.edu or 973-761-9044

Student Poster Presentations

Students wishing to give a 2011 Poster Presentation should submit their abstracts (300 word limit) on-line at the MACUB web site (www.macub.org)

If you have any questions contact Poster Co-Chairs

Dr. Edward Tall
Edward.Tall@shu.edu or 973-275-2069

or

Dr. Gerald Ruscingno
Gerald.Ruscingno@shu.edu or 973-761-9044

Student Membership

We encourage your students to become Associate Members in MACUB. Many of them will go on to graduate and professional schools. Their membership, participation and attendance at conferences such as these can enhance the experiences they include on their applications and discuss during interviews.

2011 MACUB Conference Registration Form
44th Annual MACUB Conference at Seton Hall University
Saturday, October 29, 2011

Registrations should be returned no later than October 19, 2011. Registration on the day of the conference will be $55. A separate form must be completed by each person attending the conference. Please photo copy this form for each additional registrant.

❑Dr.	❑Regular Member	❑Student Member[1]
❑Prof.	❑Full-Time Faculty	❑Member's Spouse/Guest
❑Mr.	❑Adjunct Faculty[1]	
❑Ms.		
❑ _____		

*** Name:** _____ *** School Phone:** _____
*** Department:** _____ *** Fax:** _____
*** School:** _____ *** E-Mail:** _____
*** Address:** _____
_____

***The above information may appear in a Directory of Members unless you indicate otherwise.**

Home Address: _____
_____
_____

Home Phone: _____

I prefer MACUB mailings to be sent to my:

❑ College ❑ Home[1]

[1]Student and adjunct mailings will normally be sent to your home address.

	Early Bird by 9/19	In Advance by 10/19	On-Site 10/29	
❑Regular Member	$45	$50	$55	Includes 2012 Membership dues, conference registration, continental breakfast and luncheon.
❑Student Associate Membership	$35	$35	$40	Includes 2012 Associate Membership dues, conference registration, continental breakfast and luncheon.
❑Member's Spouse/Guest	$35	$35	$40	Includes conference registration, continental breakfast and luncheon.

❑I will not be attending the Conference but enclosed is my 2011 membership dues.
❑Regular Member $20 ❑Student Member $20

Return this registration form by October 19, 2011
Please make checks payable to: MACUB
Send registration form and check to:
Dr. Paul Russo
Division of Natural Sciences & Mathematics
Bloomfield College
467 Franklin Street
Bloomfield, NJ. 07003

Conference registration fees are refundable upon written notification by **October 19, 2011.** The membership fee ($20 for regular members and $10 for student members) will be deducted. *No refunds will be given postmarked after October 19, 2011.*

The Metropolitan Association of College and University Biologists thanks the following Affiliate Members for their support:

AD Instruments
Anatomy in Clay Systems
BioPac Systems
Cengage Learning
Heyden McNeil Publishing
I. Miller Microscopes
John Wiley & Sons
McGraw Hill Publishing
Micro-Optics Precision Instruments
Pasco Scientific
Pearson Education
W. H. Freeman and Company

Please make every effort to support these affiliate members. Their participation help us to keep the registration fees at a reasonable price.

Save the Date

Saturday, October 29, 2011

The 44th Annual MACUB Conference at Seton Hall University

Dr. Edward J. Catapane
Department of Biology
Medgar Evers College
1150 Carroll Street
Brooklyn, New York 11225