



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



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How Bacteria Talk to Each Other

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*A Mixed Bag:
Bacteria That Colonize Humans*



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In This Issue:

| | |
|---|------------------------------|
| MACUB 2011-2012 Executive Board | inside cover |
| Instruction for Authors | inside cover |
| Welcome from Seton Hall University | 2 |
| Bactericidal Activity of L-carnitine Against <i>Salmonella enterica</i> Serovar Typhimurium Mouhamed Halwani, John M. Jackson, Alexander Vasilakos and Adam J. Houlihan | 3 |
| The Ability of PAS, Acetylsalicylic Acid and Calcium Disodium EDTA to Protect Against the Toxic Effects of Manganese on Mitochondrial Respiration in Gill of <i>Crassostrea virginica</i> Sherine Crawford, Kiyya Davis, Claudette Saddler, Jevaun Joseph, Edward J Catapane and Margaret A. Carroll | 7 |
| Call for Nominations for MACUB Executive Board | 15 |
| Benjamin Cummings/MACUB Student Research Grants Information | 16 |
| 44th Annual Fall MACUB Conference Presentation Information | 17 |
| MACUB Conference Registration Form | 18 |
| Affiliate Members, 44th MACUB Conference Announcement | inside back cover |



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.

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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* Serovar 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 home¹⁻⁵. 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 methionine⁸. 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 BT⁹. 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 shuttle¹⁰⁻¹². In addition, carnitine has osmoprotectant properties by acting as a compatible solute in bacteria¹³. L-carnitine also plays a role in sperm motility and energy production and helps provide a pathogen free environment in the male urogenital tract¹⁴.

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 tissue¹⁵. These compounds disrupt cell membranes and denature proteins and are effective against vegetative bacterial cells, protozoa, fungi, and enveloped viruses¹⁵. They are far less effective against spores and non-enveloped viruses¹⁵.

Quaternary ammonium compounds cause a dissociation of membrane phospholipid bilayers, directly affecting membrane permeability and resulting in leakage of cellular contents¹⁶. 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 bacteria¹⁷. 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 tuberculosis¹⁹. 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 H₂S and do not ferment lactose²⁰. 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 States², including outbreaks of multidrug resistant (MDR) *Salmonella* Typhimurium²¹. 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 10⁴ 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).

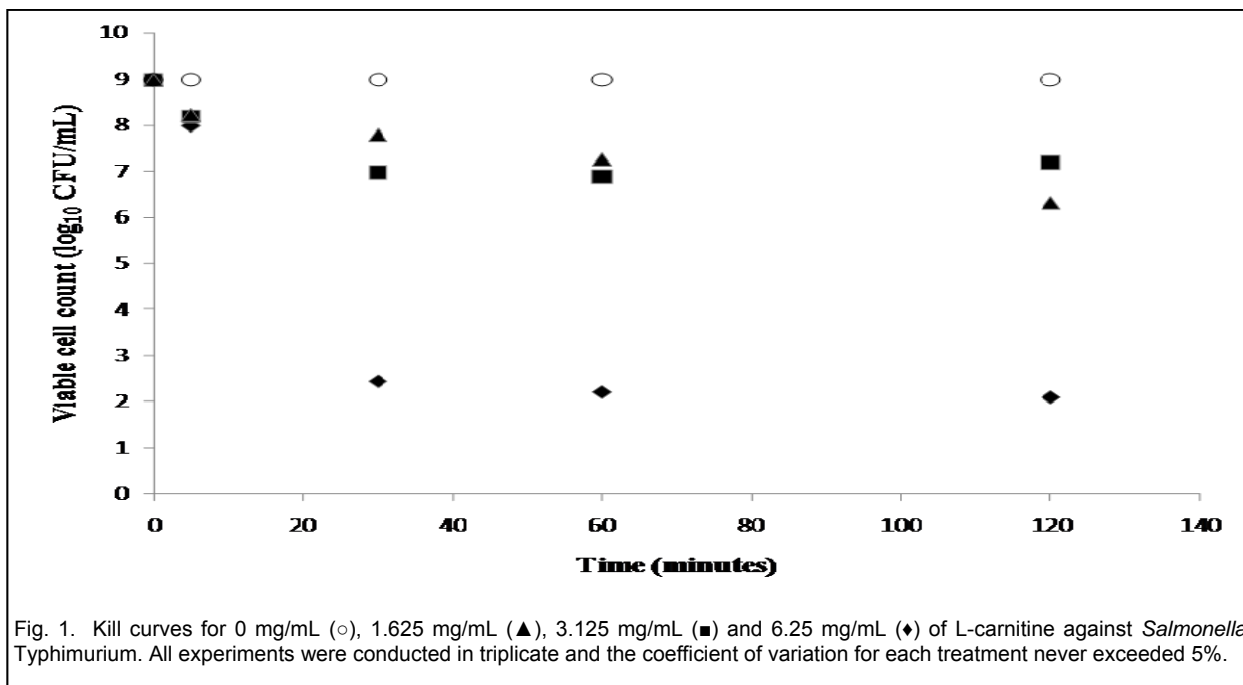
Bactericidal curves: A washed cell inoculum was created by adding an overnight culture of *Salmonella* Typhimurium (37°C, 24 hrs, with agitation) to 250 mL of fresh nutrient broth and the culture was incubated at 37°C for 150 minutes with agitation. Ten mL of the culture was added to sterile 15-mL conical 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 10⁹ 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 10⁹ cfu/mL to 451 cfu/mL. Viable cell count did not decline appreciably between 30 and 120 minutes.



Discussion

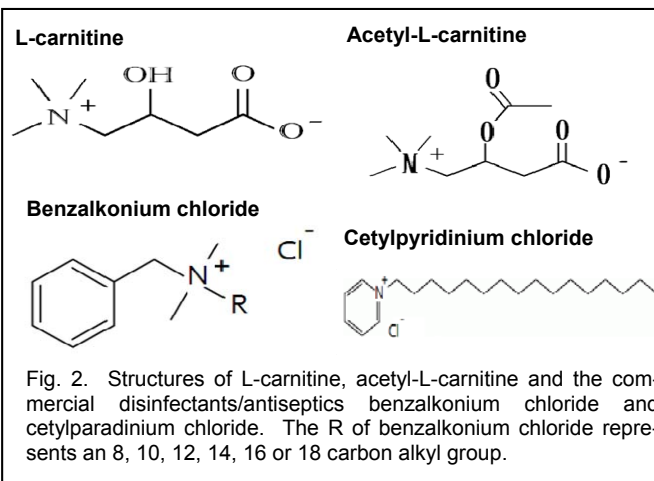
This work demonstrates that L-carnitine has antimicrobial effects against *Salmonella Typhimurium*. The MIC for L-carnitine against *Salmonella Typhimurium* was determined to be 3.125 mg/mL. To discount osmotic effects, an MIC was also determined using the simple amino acid glycine. The MIC for glycine 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.

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

- ¹Carattoli, 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.
- ²Besser, 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.
- ³Bloomfield, S.F., 2002. Significance of biocide usage and antimicrobial resistance in domiciliary environments. *Journal of Applied Microbiology* **92**: 144S–157S.
- ⁴McEwen, S.A. and P.J. Fedorka-Cray, 2002. Antimicrobial use and resistance in animals. *Clinical Infectious Disease* **34**: S93–S106.
- ⁵Wise, R. and E.J. Soulsby, 2002. Antibiotic resistance - an evolving problem. *The Veterinary Record* **151**: 371–372.
- ⁶Moellering, R.C., 1998. Antibiotic resistance: lessons for the future. *Clinical Infectious Disease* **27**: S135–140.
- ⁷Vidaver, A.K., 2002. Uses of antimicrobials in plant agriculture. *Clinical Infectious Disease* **34**: S107–110.
- ⁸Rebouche, C.J., 1991. Ascorbic acid and carnitine biosynthesis. *The American Journal of Clinical Nutrition* **54**: 1147S.
- ⁹Carter, H.E., P.K. Bhattacharyya, K.R. Weidman and G. Fraenkel, 1952. Chemical studies on vitamin B7 isolation and characterization as carnitine. *Archives of Biochemistry and Biophysics* **38**: 405–416.
- ¹⁰Kelly, G.S., 1998. L-Carnitine: therapeutic applications of a conditionally-essential amino acid. *Alternative Medicine Review* **3**(5): 345–360.
- ¹¹Yano, 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.
- ¹²Steiber, A., J. Kerner and C. Hoppel, 2004. Carnitine: a nutritional, biosynthetic, and functional perspective. *Molecular Aspects of Medicine* **25**(5-6): 455–73.
- ¹³Maras, 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.
- ¹⁴Lenzi, 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.
- ¹⁵Block, S.S., 2000. *Disinfection, Sterilization, and Preservation (5th edition)*, Lippincott, Williams & Wilkins, p 481.
- ¹⁶Thorsteinsson, T., T. Loftsson and M. Masson, July 2003. Soft Antibacterial agents. *Current Medicinal Chemistry* **10**(13): 1129–1136.
- ¹⁷Olgun, 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.
- ¹⁸Calvani, 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.
- ¹⁹Jirillo, 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.
- ²⁰Hong, 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.
- ²¹Glynn, 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 Joseph¹,
Edward J. Catapane and Margaret A. Carroll

Department of Biology, Medgar Evers College, Brooklyn, NY

¹Department 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 O₂ 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 O₂ consumption seen in Mn treated mitochondria. The study found that high levels of Mn had a toxic effect on gill mitochondrial O₂ 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 neurotoxicity 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 metabolism¹. 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 disease²⁻⁵. This Parkinson-like neurological condition first described in 1837 in two manganese ore-crushing mill workers⁶ has been referred to as Manganism⁷⁻¹⁰. Inhalation of manganese from the atmosphere is believed to be the primary cause of manganese toxicity¹¹. 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 Maneb¹²⁻¹⁶. 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 pallidus³¹⁻³⁶. 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 neurotransmission⁴¹⁻⁴³.

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 death⁴⁴⁻⁴⁶ which is in agreement with current theories on oxidative stress as a mediator of neuronal death in Parkinson's disease and other neurodegenerative diseases⁴⁷⁻⁵¹. When in excess, manganese has been shown to raise levels of reactive oxygen species⁵²⁻⁵⁵.

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 system⁵⁷. *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-aminosalicylic acid)⁵⁶, a drug that has recently been shown as a successful therapeutic agent for

Manganism⁶⁰, 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 cells⁵⁹.

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.

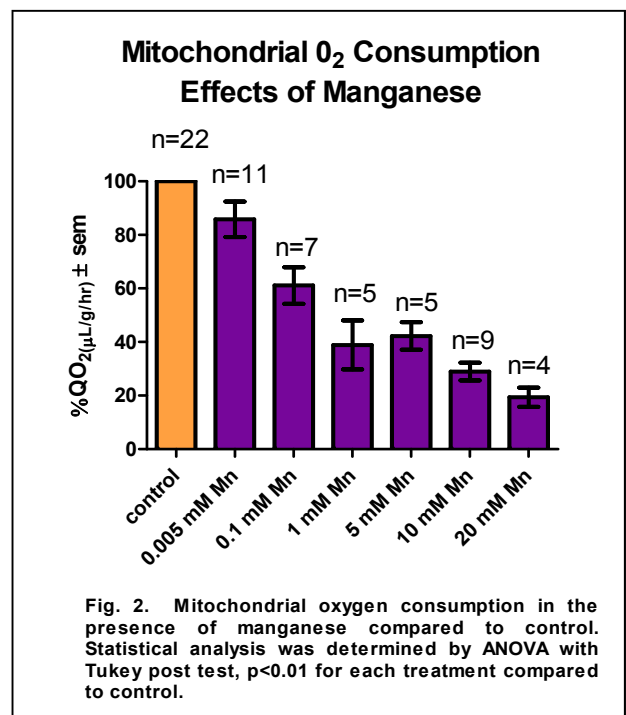
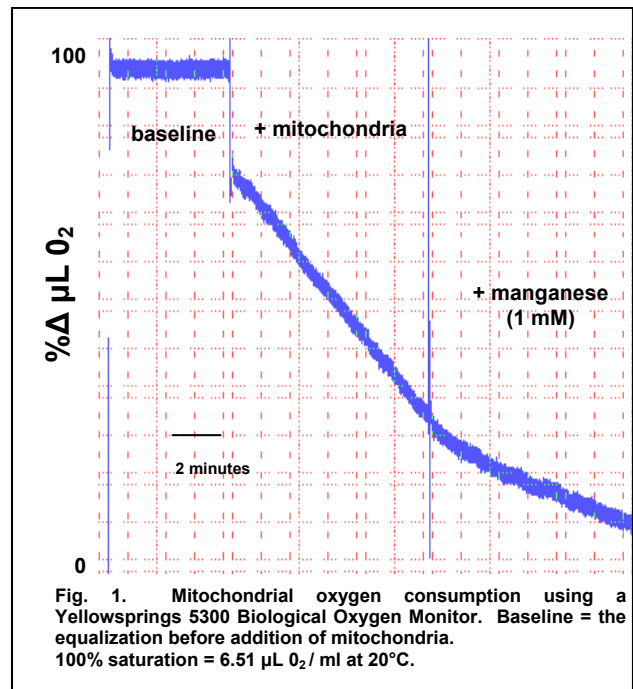
PAS and ASA were obtained from Sigma-Aldrich, St. Louis, MO. All other chemicals were obtained from Fisher Scientific, Pittsburgh, PA. Mitochondria were isolated from oyster gills. The isolation buffer consisted of 540 mM glycine, 250 mM sucrose, 2 mM EDTA, 20 mM Tris-HCl, pH 7.4, and 0.2% bovine serum 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

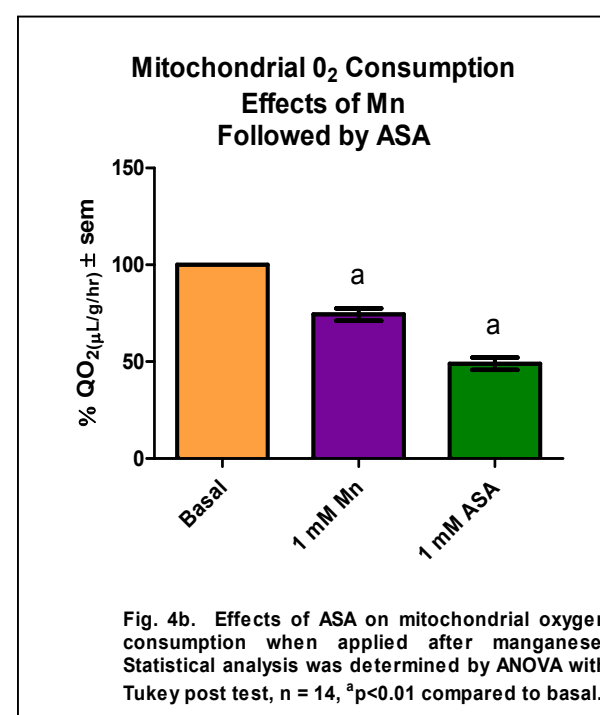
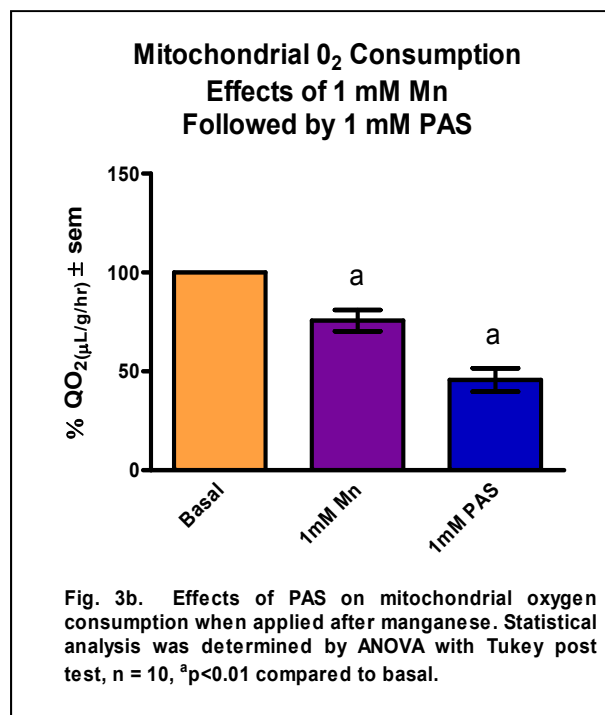
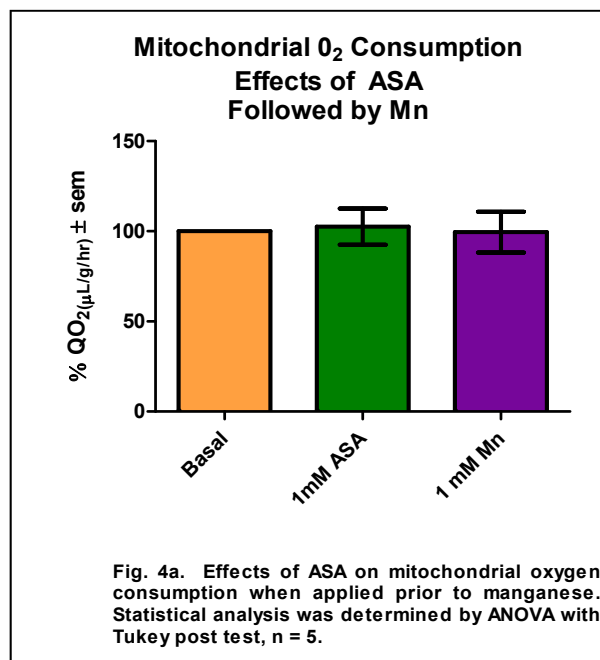
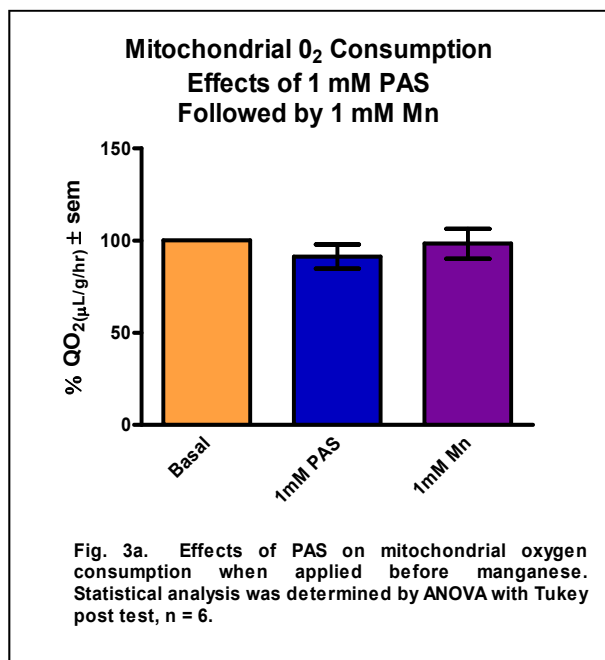
in 2 ml of ice cold EDTA-free isolation buffer. Oxygen uptake by mitochondria was measured in a Micro-Biological Oxygen Monitor with a 600 μ l micro-batch chamber (YSI, Yellow Springs, OH). Continuous data acquisition was made using a DI-700 Data Acquisition System (DATAQ Instruments, Inc., Akron, OH). 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 μ l) of isolation medium containing mitochondria was added to the chamber and basal mitochondrial respiratory rates (state 3) were measured. After 10 minutes, aliquots (20 μ l) 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.

Results

The basal oxygen utilization rate for isolated mitochondria from 22 gill samples was $6.18 \pm 0.65 \mu LO_2/hr/gm$. 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.





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.

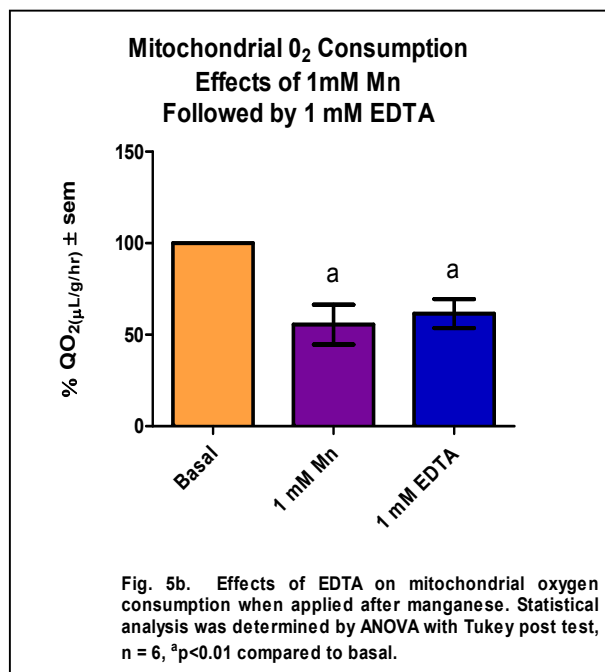
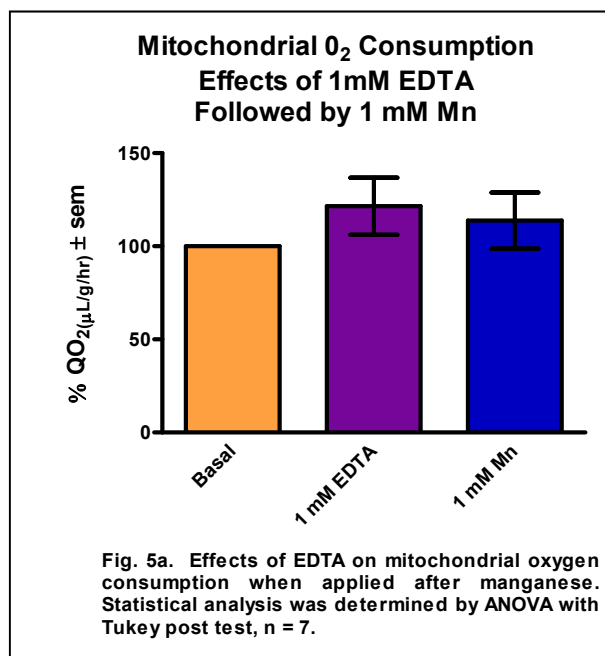
Discussion

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 Manganism²⁻⁵. 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-tetrahydropyridine (MPTP)-induced Parkinson's disease because transgenic mice who overexpressed copper/zinc superoxide dismutase were protected from the dopaminergic neuronal degeneration caused by MPTP exposure⁷³.

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 O₂ 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 lead⁷³. 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 manganese⁶⁰. ASA is known to chelate iron⁷⁴, 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 neurotoxicity, 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

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References

- ¹Cotzias, G.C., 1958. Manganese in health and disease. *Physiol. Rev.* **38**: 503-532.
- ²Calne, 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.
- ³Aschner, 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.
- ⁴Levy, B.S. and W.J. Nassetta, 2003. Neurological effects of manganese in humans: a review. *Int. J. Occup. Environ. Health* **9**: 153-163.
- ⁵Dobson, A.W., K.M. Erikson and M. Aschner, 2004. Manganese neurotoxicity. *Ann. N.Y. Acad. Sci.* **1012**: 115-128.
- ⁶Couper, J., 1837. On the effects of black oxide of manganese when inhaled into the lungs. *Br. Ann. Med. Pharmacol.* **1**: 41-42.
- ⁷Mena, I., O. Marin, S. Fuenzalida and G.C. Cotzias, 1967. Chronic manganese poisoning. Clinical picture and manganese turnover. *Neurology* **17**: 128-136.
- ⁸Barbeau, A., 1984. Manganese and extrapyramidal disorders (a critical review and tribute to Dr. George C Cotzias). *Neurotoxicology* **5**: 13-35.
- ⁹Donaldson, J., 1987. The physiopathologic significance of manganese in brain: its relation to schizophrenia and neurodegenerative disorders. *Neurotoxicology* **8**: 451-462.
- ¹⁰Gorell, 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.
- ¹¹Andersen, 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.
- ¹²National Academy of Sciences, 1973. Medical and biological effects of environmental pollutants: manganese. National Academy Press, Wash. DC, pp. 1-101.
- ¹³Meco, 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.
- ¹⁴Reidy, 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.
- ¹⁵Iregren, A., 1999. Manganese neurotoxicity in industrial exposures: proof of effects, critical exposure level, and sensitive tests. *Neurotoxicology* **20**: 315-324.
- ¹⁶Olanow, C.W., 2004. Manganese-induced parkinsonism and Parkinson's disease. *Ann. N.Y. Acad. Sci.* **1012**: 209-223.
- ¹⁷Mergler, D., 1999. Neurotoxic effects of low level exposure to manganese in human populations. *Environmental Research* **80(2)**: 99-102.
- ¹⁸Lucchini, 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.
- ¹⁹Davis, J.M., 1999. Inhalation health risks of manganese: an EPA perspective. *Neurotoxicology* **20**: 511-518.
- ²⁰Kaiser, J., 2003. Manganese: a high octane dispute. *Science* **300**: 926-928.
- ²¹Jankovic, J., 2005. Searching for a relationship between manganese and welding and Parkinson's disease. *Neurology* **64**: 2021-2028.
- ²²Dorman, 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.

- ²³Mena, 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.
- ²⁴Rosenstock, H.A., D.G. Simons and J.S. Meyer, 1971. Chronic Manganism. Neurologic and laboratory studies during treatment with levodopa. *JAMA* **217**: 1354-1358.
- ²⁵Huang, 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.
- ²⁶Barbeau, A., N. Inoué and T. Cloutier, 1976. *Advances in Neurology* **14**: 339-352.
- ²⁷Huang, 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.
- ²⁸Lu, C.S., C.C. Huang, N.S. Chum and D.B. Calne, 1994. Levodopa failure in chronic manganism. *Neurology* **44**: 1600-1602.
- ²⁹Koller, 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.
- ³⁰Cersosimo, M.G. and W.C. Koller, 2006. The diagnosis of manganese-induced parkinsonism. *NeuroToxicology* **27**: 340-346.
- ³¹Eriksson, 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.
- ³²Brenneman, 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.
- ³³Nagatomo, 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.
- ³⁴Newland, M.C., 1999. Animal models of manganese's neurotoxicity. *Neurotoxicology* **20**: 415-432.
- ³⁵Pal, P.K., A. Samii and D.B. Calne, 1999. Manganese neurotoxicity: a review of clinical features. *Neurotoxicology* **20**: 227-238.
- ³⁶Baek, 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.
- ³⁷Parenti, M., C. Flauto, E. Parati, A. Vescovi and A. Gropetti, 1986. Manganese neurotoxicity: effects of L-DOPA and pargyline treatments. *Brain Res.* **367**: 8-13.
- ³⁸Eriksson, 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.
- ³⁹Vescovi, 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.
- ⁴⁰Sistrunk, 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.
- ⁴¹Neff, 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.
- ⁴²Hornykiewicz, O., 1972. Dopamine and extrapyramidal motor function and dysfunction. *Res. Publ. Assoc. Res. Nerv. Ment. Dis.* **50**: 390-415.
- ⁴³Graham, D.G., 1984. Catecholamine toxicity: a proposal for the molecular pathogenesis of manganese neurotoxicity and Parkinson's disease. *Neurotoxicology* **5**: 83-95.
- ⁴⁴HaMai, D. and S.C. Bondy, 2004. Oxidative basis of manganese neurotoxicity, *Annals of the New York Academy of Sciences* **1012**: 129-141.
- ⁴⁵Anantharam, 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.
- ⁴⁶Latchoumycandane, C., V. Anantharam, M. Kitazawa, Y. Yang, A. Kanthasamy and A.G. Kanthasamy, 2005. Protein kinase C delta is a key downstream mediator of manganese-induced apoptosis in dopaminergic neuronal cells. *Journal of Pharmacology and Experimental Therapeutics* **313**: 46 - 55.
- ⁴⁷Fahn, S. and G. Cohen, 1992. The oxidant stress hypothesis in Parkinson's disease: evidence supporting it. *Annals of Neurology* **32**: 804 - 812.
- ⁴⁸Albers, 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.

- ⁴⁹Schulz, J.B., J. Lindenau, J. Seyfried and J. Dichgans, 2000. Glutathione, oxidative stress and neurodegeneration. *European Journal of Biochemistry* **267**: 4904 - 4911.
- ⁵⁰Dawson, T.M. and V.L. Dawson, 2003. Molecular pathways of neurodegeneration in Parkinson's disease. *Science* **302**: 819-822.
- ⁵¹Emerit, J., M. Edeas and F. Bricaire, 2004. Neurodegenerative diseases and oxidative stress. *Biomedicine and Pharmacotherapy* **58**: 39 - 46.
- ⁵²Ali, 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.
- ⁵³Shi, 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).
- ⁵⁴Brouillet, E.P.L., U. Shinobu, F. McGarvey, F. Hochberg and M.F. Beal, 1993. Manganese injection into the rat striatum produces excitotoxic lesions by impairing energy metabolism. *Experimental Neurology* **120**: 89 - 94.
- ⁵⁵Archibald, F.S. and C. Tyree, 1987. Manganese poisoning and the attack of trivalent manganese upon catecholamines. *Archives of Biochemistry and Biophysics* **256**: 638 - 650.
- ⁵⁶King, 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.
- ⁵⁷Carroll, 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.
- ⁵⁸Martin, 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.
- ⁵⁹Nelson, M., T. Huggins, R. Licorish, M.A. Carroll and E.J. Catapane, 2010. Effects of p-Aminosalicylic acid on the neurotoxicity of manganese 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.
- ⁶⁰Jiang, 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.
- ⁶²Anantharam, 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.
- ⁶³Stredrick, 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.
- ⁶⁴Fahn S and G. Cohen. 1992. The oxidant stress hypothesis in Parkinson's disease: evidence supporting it. *Ann Neuro* **132**: 804-812.
- ⁶⁵Schulz, J.B., Lindenau, J., Seyfried, J., Dichgans, J., 2000. Glutathione, oxidative stress and neurodegeneration. *Eur. J. Biochem.* **267**: 4904 -4911.
- ⁶⁶Dawson, T.M. and V.L. Dawson, 2003. Molecular pathways of neurodegeneration in Parkinson's disease. *Science* **302**: 819-822.
- ⁶⁷Emerit, J., M. Edeas and F. Bricaire, 2004. Neurodegenerative diseases and oxidative stress. *Biomedicine and Pharmacotherapy* **58 (1)**: 39-46.
- ⁶⁸Halliwell, B., 1992. Reactive oxygen species and the central nervous system. *J. Neurochem.* **59**: 1609-1623.
- ⁶⁹Lotharius, 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.
- ⁷⁰Cantuti-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.
- ⁷¹Dauer, W. and S. Przedborski, 2003. Parkinson's Disease: Mechanisms and Models. *Neuron* **39 (6)**: 889-909.
- ⁷²Przedborski, 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-1,2,3,6-tetrahydropyridine-induced neurotoxicity. *J Neurosci* **12**:1658-1667.
- ⁷³Gervasio A., S. Lamas and J. Hussein, 2006. EDTA chelation therapy meets evidence-based medicine *Complementary Therapies in Clinical Practice* **12(3)**: 213-215.
- ⁷⁴Schwarz, 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**

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**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**

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**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 Ruscigno
Gerald.Ruscigno@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.

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| <input type="checkbox"/> Dr. <input type="checkbox"/> Prof. <input type="checkbox"/> Mr. <input type="checkbox"/> Ms. <input type="checkbox"/> _____ | <input type="checkbox"/> Regular Member <input type="checkbox"/> Full-Time Faculty <input type="checkbox"/> Adjunct Faculty ¹ | <input type="checkbox"/> Student Member ¹ <input type="checkbox"/> Member's Spouse/Guest |
|--|--|--|

| | |
|---------------------|-----------------------|
| * Name: _____ | * School Phone: _____ |
| * Department: _____ | * Fax: _____ |
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| * Address: _____ | |

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

Home Address: _____

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I prefer MACUB mailings to be sent to my:

- College Home¹

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

| | Early Bird by 9/19 \$45 | In Advance by 10/19 \$50 | On-Site 10/29 \$55 | |
|---|-------------------------------|--------------------------------|--------------------------|---|
| <input type="checkbox"/> Regular Member | | | | Includes 2012 Membership dues, conference registration, continental breakfast and luncheon. |
| <input type="checkbox"/> Student Associate Membership | \$35 | \$35 | \$40 | Includes 2012 Associate Membership dues, conference registration, continental breakfast and luncheon. |
| <input type="checkbox"/> 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.*

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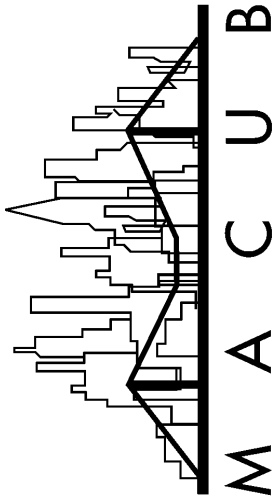
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Save the Date

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