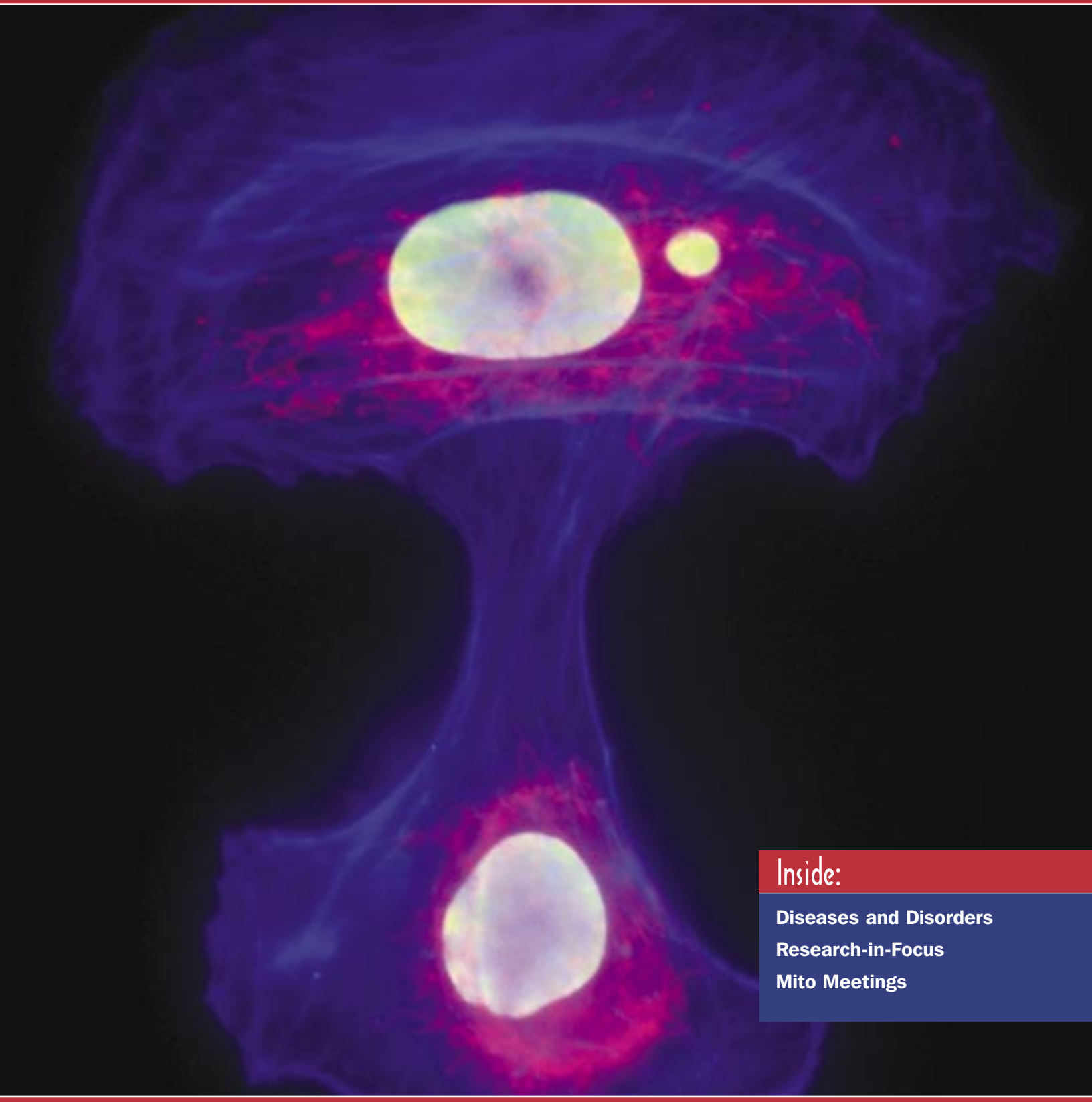
MitoMatters

The Official Newsletter of the Mitochondria Research Society



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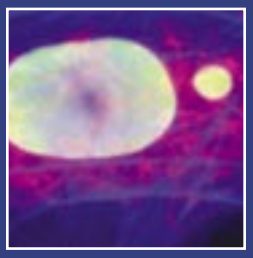

Dear Colleagues:

It's a pleasure to inform you that MRS together with The Mitochondrial Medicine Society and United Mitochondrial Disease Foundation is organizing the "Mitochondrial Medicine 2004" meeting in Pittsburgh, PA, at Westin Convention Center Hotel from August 4 to 7, 2004. Please visit www.umdf.org for more details.

We are also happy to report that you can now renew your membership online on a secure Plug and Pay server. Please visit www.mitoresearch.org. We are streamlining the dates for yearly memberships and are requesting everyone to renew their membership as soon as possible. The membership now begins January 1 and ends December 31 each year. You will also find the membership application/renewal form with this newsletter. If you do not wish to renew your membership online, please send check or credit card information for membership dues along with the form as soon as possible. With your renewal you will continue to receive an uninterrupted subscription to the *Mitochondrion* journal and this newsletter, as well as other membership benefits. Please note that only the members who have paid their dues will receive the journal. Please register at our website (www.mitoresearch.org). We intend to distribute this newsletter electronically. If you do not register at our website, you may not receive the newsletter.

Sincerely,

Managing Editors

Front cover image: *Muntjac skin fibroblasts labeled with a mouse monoclonal anti-histone antibody and visualized with green-fluorescent Alexa Fluor® 500 goat anti-mouse IgG antibody. F-actin was labeled with blue-fluorescent Alexa Fluor® 350 phalloidin, and mitochondria were stained with red-fluorescent MitoTracker® Red CMXRos. Image contributed by Michael Janes, Molecular Probes Inc.*

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Statin-Associated Myopathy Is Associated With Impaired β-Oxidation of Fatty Acids

Paul S. Phillips, MD, Director, Interventional Cardiology
Scripps Mercy Hospital, 4077 Fifth Avenue (mer 74), San Diego, CA 92103
Phillips.paul@scrippshealth.org

HMG-CoA reductase inhibitors, or statins, are the most popular cholesterol-lowering drugs. These drugs have met with great success in demonstrating 30% reductions in cardiovascular endpoints. They have been tested in over 80,000 patients without serious morbidity, and they are routinely touted as safer than aspirin. Due to this great success without demonstrated toxicity, statins are now the No. 1 class of new prescriptions worldwide. They are taken by over 15 million Americans, and current recommendations would advise over 36 million should be taking this therapy (ATP 3, 2001).

The withdrawal of cerivastatin (Baycol) from the U.S. market in August 2001 created considerable surprise and concern. Cerivastatin was withdrawn because of an unacceptable rate of fatal rhabdomyolysis, or muscle toxicity, which ultimately resulted in over 100 deaths. This unanticipated incidence of rhabdomyolysis has highlighted our ignorance of the cause of myotoxic reactions to lipid-lowering therapies. Other statins cause nonfatal elevations of creatine kinase (CK) to over 10 times normal in only 1/10,000 patient years of statin use (Gruer, 1999). However, milder muscle complaints such as aching are common. This leaves physicians and patients with significant concerns. Therefore, only half of the patients who should be receiving lipid-lowering therapies are receiving them. It is imperative that we understand myotoxicity reactions more completely in order to appropriately treat hyperlipidemias.

We have attempted to understand the etiology of muscle reactions to lipid-lowering therapy by a series of experiments. In order to define the syndrome of statin-myotoxicity, we evaluated 54 patients with normal CK who believed that their muscle complaints were due to statin therapy via double-blind crossover methodologies (Phillips P, 2004a). Two-thirds of the subjects correctly identified the blinded statin arm. Many developed measurable weakness on statins that reversed when therapy was discontinued. Five were biopsied on statins, and all demonstrated myopathology including increased lipid on oil red O stains, cox-negative staining fibers and ragged red fibers. Three patients who could not identify the blinded statin arm had muscle biopsies, and none of these had abnormalities. Preliminary results were sufficient to prove that some patients with muscle complaints on statin therapy suffer myopathy with a normal creatine kinase (Phillips P, 2002a).

We next evaluated 11 subjects who had previously suffered statin-induced rhabdomyolysis or myositis. Half of these patients never made a full recovery from their myositis event. Biopsies of these subjects revealed the same abnormal depositions of neutral lipid we had seen in the normal CK myopathy group. Cox-negative staining fibers and ragged red fibers as well as mitochondrial inclusions were frequently noted. Similarities between the myositis and the normal CK groups mentioned above went beyond this pathology. The preponderance of patients in each group were hypertriglyceridemic, and subjects in both groups excreted abnormal urinary organic acids when on statins (Phillips P, 2002b).

Since half of our post-myositis patients suffered long-term sequelae after their index toxic event, we searched for information about recovery from this process. There is little or no information available from review of the literature or from the FDA. We designed a public information website with surveys for patients suffering muscle reactions to statins to answer this question.

Data from over 1,000 patients with rhabdomyolysis (81) and other myopathic reactions to statins (972) have been analyzed (Kordas, 2004). Data from this website confirmed that 50% of rhabdomyolysis survivor respondents did not fully recover from their muscle toxicity. Furthermore, the mean triglyceride level was 341 mg among respondents in both categories. This unusual elevation in triglycerides suggests that the patients with muscle toxicity are a special subgroup among patients with hyperlipidemia. This correlated with our smaller clinical experience where the majority of subjects suffering muscle toxicity had baseline elevations in triglycerides. This has suggested

that in some patients, hypertriglyceridemia is a marker for an underlying defect in fatty acid oxidation that may be exacerbated by lipid-lowering therapies.

The pathology and the elevated fasting triglyceride levels in statin myopathy are similar to findings in carnitine palmitoyl transferase (CPT) deficiency (Bank, 1975). CPT deficient subjects have an abnormal fasting respiratory exchange ratio (RER) secondary to impaired fatty acid oxidation. Therefore, we performed experiments to determine whether RER was affected by statin toxicity. Six controls with a normal fasting RER of 0.76 ± 0.03 increased their RER to 0.86 ± 0.06 after six weeks of statin use (p=0.0001) (Phillips C, 2004a). Even more interesting, the 11 subjects who had previously suffered statin-associated myositis or rhabdomyolysis had a fasting RER of 0.92 ± 0.07 when tested off statins (Phillips C, 2004b). This suggests a significant impairment in fatty acid oxidation in these subjects. It is unclear if this defect preceded statin exposure or resulted from that exposure.

The clinical, biochemical and pathological similarities between statin-induced normal CK myopathy and patients suffering symptoms after statin-induced rhabdomyolysis or myositis support a defect in the β-oxidation of fatty acids. We have recently started a series of experiments in myocytes cultured from statin myopathy patients. Results from these cell cultures will allow us to determine if the defect in fat oxidation lies within the mitochondrion or among the cellular lipid transport proteins. Identification of this defect will provide insight into the diagnosis and treatment of myotoxic reactions induced by various lipid-lowering therapies.

Based on this body of work we are able to state the following hypotheses: There are pathological, biochemical and physiological homologies among the various forms of myotoxicity due to lipid-lowering therapy from myopathy to rhabdomyolysis. These similarities suggest impaired β-oxidation of fatty acids is the common denominator among subjects vulnerable to these reactions. Myotoxicity seems to occur in selected hypertriglyceridemic patients with abnormal fatty acid oxidation who are exposed to most lipid-lowering therapies. While the defect in beta oxidation of fatty acids remains obscure at this time, current studies of fatty acid oxidation in myocytes cultured from myotoxic patients may soon identify the location of that defect.

References:

ATP 3: Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Available at htpp://www.nhlbi.nih.gov.

Bank, W.J., DiMauro, S., Bonilla, E., Capuzzi, D.M., Rowland, L.P. 1975. A disorder of muscle lipid metabolism and myoglobinuria—absence of carnitine palmitoyl transferase. *NEJM* 292:443–449.

Gruer, P.J.K., Vega, J.M., Mercuri, M.F., Dobrinska, M.R., and Tobert, J.A. 1999. Concomitant use of cytochrome P450 3A4 inhibitors and simvastatin. *Am J Cardiol* 84:811–815.

Kordas, K.C., Phillips, P.S., Golomb, B.A. 2004. Clinical characteristics of 1053 patients with statin associated muscle complaints [abstract]. (Accepted—5th Annual Conference on Arteriosclerosis, Thrombosis and Vascular Biology, San Francisco.)

Phillips, P.S., Maggasy, G., Sikand, H., Gray, N.L., Puhek, L.M., Thomas, R.G., and the Scripps Clinical Research Center. 2004. Statin associated myopathy with normal creatine kinase—results of a pilot trial. In preparation.

Phillips, P.S., Haas, R.H., Bannykh, S., Hathaway, S., Gray, N.L., Kimura, B.J., Vladutiu, G.D., England, J.D.F., and the Scripps Mercy Clinical Research Center. Statin associated myopathy with normal creatine kinase. *Ann Int Med* 137:581–585.

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Phillips, C.T., Gray, N.L., Puhek, L.M., McDonald, F.G., Sullivan, M.J., Phillips, P.S. 2004. Basal respiratory exchange ratio is altered with statin use in normals [abstract]. *J Am Coll Cardio* 43 (suppl A):233a.

Phillips, P.S., Kimura, B.J., Kelley, R., Thomas, R.G. 2003. Feasibility of using the internet to perform medical research: observations from a statin-myopathy public information web site. *Circulation* 107: e7001–e7039.

Research-in-Focus

1. The mitochondrial proteome: The complete complement of proteins present in a mammalian mitochondrion is unknown. Estimates based on comparison to lower eukaryotes and eubacterial relatives have suggested that the organelle contains approximately 1200 proteins. Using a combined proteomics/genomics approach, Mootha and colleagues have identified 591 proteins in mouse mitochondria from brain, heart, kidney and liver, including 163 not previously associated with mitochondria. The authors also found tissue-specific differences in organelle composition. This work, along with data from expression profile studies, provides a groundwork for the understanding of how mitochondria biology is regulated.

Mootha,V.K., Bunkenborg, J., Olsen, J.V., Hjerrild, M., Wisniewski, J.R., Stahl, E., Bolouri, M.S., Ray, H.N., Sihag, S., Kamal, M., Patterson, N., Lander, E.S., Mann, M. 2003. Integrated analysis of protein composition, tissue diversity, and gene regulation in mouse mitochondria. *Cell* 115: 629–640.

2. mtDNA and climate control: mtDNA sequence variants are very common in human populations, and haplotypes (mtDNA sequences) have often been used to characterize indigenous populations. A recent paper by Ruiz-Pesini and colleagues suggests that more than genetic drift has influenced haplotype distribution. The analysis of 104 complete haplotypes from around the world showed that the relative frequency and amino acid conservation of internal branch replacement mutations increased as populations moved from tropical Africa to temperate Europe and to Arctic Siberia. This suggests that mutations in amino acids that caused some degree of energy inefficiency were selected to adapt to colder climates.

Ruiz-Pesini, E., Mishmar, D., Brandon, M., Procaccio, V., Wallace, D.C. 2004. Effects of purifying and adaptive selection on regional variation in human mtDNA. *Science* 303: 223–226.

3. A xenomitochondrial mouse: The technology of transferring mtDNA from one cell line to another, the recipient being usually an mtDNA-less cell line, has been extensively used to study various mtDNA mutations that are associated with diseases. McKenzie and co-workers have taken this approach one step further, by recently generating a homoplasmic mouse line with foreign mtDNA. They introduced mtDNA from two different mouse species, Mus spretus and Mus dunni, into ES cells of the common laboratory mouse Mus musculus. Chimeras and G1 offspring were successfully obtained. This study confirms the feasibility of producing mitochondrial defects in mouse models by using the xenomitochondrial approach.

Mckenzie, M., Trounce, I.A., Cassar, C.A., Pinkert, C.A. 2004. Production of homoplasmic xenomitochondrial mice. *Proc. Natl. Acad. Sci. USA* 101: 1685–1690.

4. Protecting the brain in times of stress: Hypoxia/reoxygenation cycles, like those associated with stroke or heart surgery, can lead to increased oxidative stress and ultimately mitochondria-mediated apoptotic cell death. Yermolaieva and colleagues recently demonstrated that adenovirus-mediated overexpression of methionine sulfoxide reductase type A (MSRA) protects neuronal-like cells against hypoxia/reoxygenation, mitochondrial dysfunction and cell death. These observations provide the basis for the development of therapeutic approaches using MSRA and other antioxidant enzymes in ischemic heart and brain.

Yermolaievsa, O., Xu, R., Schinstock, C., Brot, N., Weissbach, H., Heinimann, S.H., Hoshi, T. 2004. Methionine sulfoxide reductase A protects neuronal cells against brief hypoxia/reoxygenation. *Proc. Natl. Acad. Sci. USA* 101: 1159–1164.

5. What really constitutes the pore?: Opening of the mitochondrial permeability transition pore (MPT) has been implicated in mitochondrial dysfunction and cell death. The MPT was thought to be formed by the voltage-dependent anion channel, members of the Bcl-2 family, cyclophilin D and the adenine-nucleotide translocator (ANT). However, recent results by Kokoszka and colleagues suggest that the ANT is not an essential component of the MPT. They have generated mice in which the two isoforms of ANT were inactivated in the liver. Mitochondria lacking ANT could still undergo permeability transition, although requiring more calcium. In addition, hepatocytes from those animals remain competent in responding to apoptotic stimuli.

Kokoszka, J.E., Waymire, K.G., Levy, S.E.; Sligh, J.E., Cai, J., Jones, D.P., MacGregor, G.R., Wallace, D.C. 2004. The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. *Nature* 427: 461–465.

Mito Meetings

JUNE 30–JULY 4, 2004

6TH EUROMIT MEETING

Contact: Jan Smeitink, MD, PhD
Nijmegen Center for Mitochondrial Disorders
Department of Pediatrics
University Medical Center Nijmegen
Geert Grooteplein 10
P.O. BOX 9101
6500 HB Nijmegen
The Netherlands
E-mail: j.smeitink@cukz.umcn.nl
www.euromit.org

AUGUST 4–7, 2004

MITOCHONDRIAL MEDICINE 2004

This meeting is hosted by the United Mitochondrial Disease Foundation (UMDF) in cooperation with Pittsburgh Mercy Health System, Mitochondria Research Society and Mitochondrial Medicine Society.
Contact: Rajiv Varma, MD, FAAP or
Kara Strittmatter
UMDF
8085 Saltsburg Road, Suite 201
Pittsburgh, PA 15239
E-mail: Kara Strittmatter
Tel: 412-793-8077
Fax: 412-793-6477
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AUGUST 21–26, 2004

13TH EUROPEAN BIOENERGETICS CONFERENCE 2004

Palazzo dei Congressi
Pisa, Italy
The conference is organized by the Italian Bioenergetics and Biomembranes Group. For more information contact Bruno Andrea Melandri (Chairman of the Local Scientific Committee), Dip. di Biologia, Università di Bologna, Italia. E-mail: melandri@alma.unibo.it. Giancarlo Solaini (member of the Local Scientific Committee and chairman of the Local Organizing Committee), Scuola Superiore di Studi Universitari e di Perfezionamento S. Anna, Pisa, Italia.
E-mail: gsolaini@sssup.it
www.ebec2004.sssup.it

SEPTEMBER 15–18, 2004

CROSS TALK BETWEEN NUCLEUS AND ORGANELLES

Naples, Italy
Contact: Luigi Del Giudice
E-mail: delgiudi@iigb.na.cnr.it

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Membership benefits include:

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3. MRS member directory
4. Reduced rate of job posting at MRS website
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Please specify

Current research: _____

_____

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Buffalo, NY 14221
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