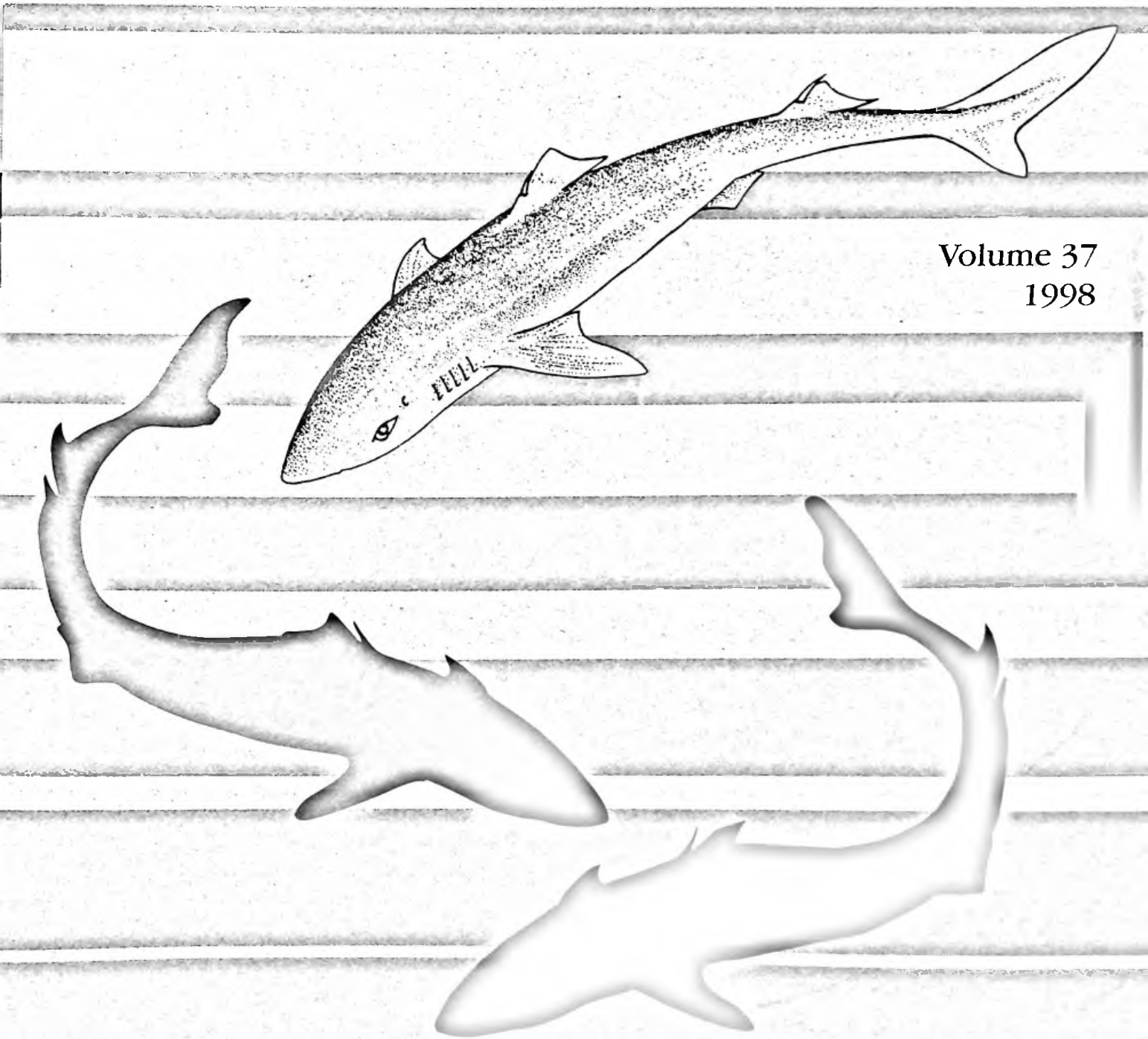
THE BULLETIN

Mount Desert Island Biological Laboratory

Celebrating 100 Years of Research and Education



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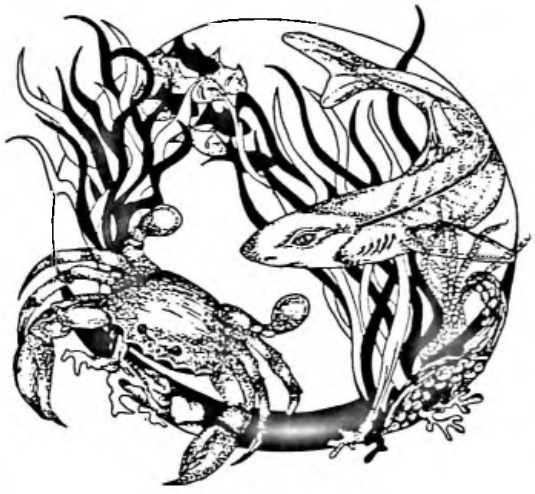

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THE MOUNT DESERT ISLAND BIOLOGICAL LABORATORY

RESEARCH AND EDUCATION IN THE BIOLOGY OF MARINE ANIMALS

INTRODUCTION

The Mount Desert Island Biological Laboratory (MDIBL) is an independent non-profit biological station located on the north shore of Mount Desert Island, overlooking the gulf of Maine about 120 miles northeast of Portland near the mouth of the Bay of Fundy. The island, well known for Acadia National Park, provides a variety of habitats including shallow and deep saltwater, a broad intertidal zone, saltwater and freshwater marshes, freshwater lakes and streams, forests and meadows.

The Laboratory is the largest cold water research facility in the Eastern United States, and its unique site provides an outstanding environment for studying the physiology of marine and freshwater flora and fauna. During 1996, the scientific personnel included 50 principal investigators and 109 associates, representing 43 institutions in 25 states and 3 European countries.

HISTORY AND ORGANIZATION

MDIBL was founded in 1898 at South Harpswell, Maine by J.S. Kingsley of Tufts University. Its present site at Salsbury Cove was donated by the Wild Gardens of Acadia, and relocation was completed in 1921. The Wild Gardens of Acadia, a land-holding group headed by George B. Dorr and John D. Rockefeller, Jr., who was instrumental in the founding of Acadia National Park.

The Laboratory was incorporated in 1914 under the laws of the State of Maine as a non-profit scientific and educational institution. Founded as a teaching laboratory, MDIBL is now a center for marine research and education that attracts investigators and students from across the U.S. and around the world. Since the pioneering work of H.W. Smith, E.K. Marshall and Roy P. Forster on various aspects of renal and osmoregulatory physiology of local fauna, the Laboratory has become known worldwide as a center for investigations in electrolyte and transport physiology, developmental biology and electrophysiology.

The Mount Desert Island Biological Laboratory is owned and operated by the Board of Trustees and Members of the Corporation; at present, there are 429 members. Officers of the Corporation - Chair, Vice-Chair, Director, Secretary, Treasurer, Clerk - and an Executive Committee are elected from among the Trustees. The Chair and Executive Committee oversee and promote long range goals of the Laboratory. The Director, with the aid of a full-time Administrative Director, staff and a Scientific Advisory Committee is responsible for implementing the scientific, educational and public service activities of the Laboratory.

NIEHS TOXICOLOGY CENTER

In 1985, with the support of the National Institute of Environmental Health Sciences (NIEHS), MDIBL established a center dedicated to the study of the toxic effects of heavy metals and other environmental pollutants that pose an increasing health risk to humans and a threat to the marine environment. The focus of The Center for Membrane Toxicity Studies (CMTS), it is the use of the marine animals like the shark, the founder and the skate to define sites of action for metals such as mercury and cadmium that enter the environment due to improper disposal of industrial waste and as a component of some pesticides. The effects of these pollutants are widespread in the human body, with affected organs including the brain, the kidney, the liver, the gastrointestinal tract and the reproductive system. The goal of the CMTS is to identify the molecular targets for toxic substances and to provide the scientific basis for the development of treatments for heavy-metal intoxication. Inquiries concerning the center are welcome.

APPLICATIONS AND FELLOWSHIPS

Research space is available for the entire summer season (June 1 - September 30) or a half-season (June 1 - July 31 or August 1 - September 30). Applications for the coming summer must be submitted by February 1st each year. Investigators are invited to use the year-round facilities at other times of the year, but such plans should include prior consultation with the MDIBL office concerning available facilities and specimen supply.

A number of fellowships and scholarships are available to research scientists, undergraduate faculty and students, and high school students. These funds may be used to cover the cost of laboratory rent, housing and supplies. Stipends are granted with many of the student awards. Applicants for fellowships for the coming summer research period are generally due in January.

For further information on applications and fellowships/scholarships,
please contact:

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ACKNOWLEDGEMENTS

The Mount Desert Island Biological Laboratory is indebted to the National Science Foundation and National Institutes of Health for substantial support. Funds for building renovations and new construction continue to permit the Laboratory to expand and upgrade its research and teaching facilities. Individual research projects served by the Laboratory are funded by private and government agencies, and all of these projects have benefited from the NSF and NIH grants to the Laboratory. For supporting our educational initiative, MDIBL acknowledges the Cserr/Grass Foundation, Milbury Fellowship Fund, American Heart Association - Maine Affiliate, Mr. Robert E. Blum, Bodil Schmidt-Nielsen Fellowship Fund, Maine Community Foundation, NSF - Research Experience for Undergraduates and The William Randolph Hearst Foundation Young Scholar Program for High School students and many local businesses and individuals.

THE FOURTH ANNUAL MDIBL ENVIRONMENTAL HEALTH SCIENCES SYMPOSIUM: HEALTH EFFECTS OF METALS IN MARINE AND FRESHWATER ENVIRONMENTS

INTRODUCTION: HISTORY OF GLOBAL METAL POLLUTION

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Metals are integral components of our environment, economy, culture, and of life itself. Metals comprise approximately 80 of the 110 known elements, and about 15 of these metals are essential for life. However, both the essential and nonessential metals can be toxic to living organisms. Human activity has greatly altered the natural biological and geological cycles that redistribute metals in the environment, and has created the opportunity for exposure to hazardous levels of the various elements. Historically, the domestication of fire provided perhaps the first opportunity for significant human exposure to metals and other air pollutants, and for disruption of the normal biological and geological metal cycles. The next major event in the history of global metal pollution was the discovery of mining and metal-working technique in ancient times. Metal extraction, purification, and utilization became and continues to be a central feature of human economy and culture. The rise and fall of ancient civilizations was inextricably linked to their ability to harness and exploit the metal elements in their environment. For example, the power of the Roman Empire is often attributed to technological advancements made possible by the mining and refining of large quantities of lead, copper, zinc, mercury, tin and arsenic. However, the uncontrolled smelting of large quantities of ore in open fires resulted in significant emissions of trace metals to the atmosphere, and in high morbidity and mortality in workers employed in these trades. Some researchers have speculated that the fall of the Roman Empire may be related at least in part to metal toxicity, resulting from the consumption of wine and other food products contaminated with high levels of lead, arsenic and mercury.

Although mining practices during ancient times had significant impact on global metal pollution, it was the development of large furnaces with tall stacks during the 11th century, and the Industrial Revolution in the modern era, that have drastically increased global metal emissions. Records of metal pollution dating back to ancient times are preserved in several types of natural deposits, including polar ice caps, aquatic sediments, and ombrogenic bogs. These records document how modern human activity has increased metal emissions and deposition in the environment, but more importantly, they also show that emissions of toxic metals have slowed or even decreased in very recent times, from about 1980 to the present. Although this trend is encouraging, the continued high demand for metals, particularly in developing countries, indicates that emissions will continue at a high rate. It is imperative that we continue to monitor and control emissions of metals into the environment, and that we continue to monitor the adverse effects on our ecosystems as well as in exposed human populations. In addition, we need to generate quantitative information regarding dose and tissue levels, and biological effects associated with these dose levels, particularly at the cellular and molecular level. This information is critical for establishing appropriate biomarkers of toxicity, and in developing guidelines for preventative or therapeutic interventions.

The state-of-the-art presentations in this symposium addressed many of these central questions, starting from an ecosystem perspective and progressing to specific molecular interactions. The conference was sponsored in part by the National Institute of Environmental Health Sciences' Center for Membrane Toxicity Studies at the MDIBL (ES03828), and the MDIBL.

CHEMICAL AND BIOLOGICAL INTERACTIONS AFFECTING HEAVY METAL FATE AND EFFECTS IN NEW BEDFORD HARBOR, MA

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The fate and effects of toxic metals in marine sediments depend on a combination of the physical, chemical and biological conditions encountered in any given environment. These conditions may vary dramatically, both spatially and temporally, in response to factors ranging from seasonal changes to storm events, to human activities such as dredging or remediation efforts. This paper will describe our research program, designed to evaluate the interrelationships between the microbial community and pollutants in the New Bedford Harbor area, an EPA designated Superfund site. Our research has focused on a number of areas including, a) fluxes of contaminant metals between sediments and the overlying water, b) effects of benthic biological activity on fluxes and subsequent bioavailability, c) changes in microbial diversity in response to different concentrations of metals, and, d) use of the microbial community as a biomarker of contaminant exposure. Our research has shown that a significant flux of metals to the water column is mediated by benthic biological activity, and that microbial communities may be a responsive marker of contaminant stress. This paper will describe how the combination of biogeochemical studies and use of molecular tools can be used to improve our understanding of the fate and effect of heavy metals on aquatic ecosystem health.

METAL DETOXIFICATION MECHANISMS IN PHYTOPLANKTON: IMPLICATIONS FOR BIOACCUMULATION

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Eukaryotic and prokaryotic microalgae employ a number of detoxification mechanisms to ameliorate heavy metal toxicity. Many metals, including Cd, Cu, Zn and Pb, induce intracellular phytochelatin production in eukaryotic algae whereas prokaryotic algae make metallothioneins in response to Cd and Zn as well as some other metals. Once metals are chelated intracellularly, they may be stored in a vacuole or exported from the cell. The marine diatom, *Thalassiosira weissflogii*, exports Cd and phytochelatin into the culture medium when exposed to high Cd concentrations. *Synechococcus* species have been shown to release specific Cu-chelators in order to buffer free Cu ion concentrations to below levels that are toxic (Moffett and Brand, Limnol. Oceanogr., 41:388, 1996). Various field studies have provided evidence that at least a few of these detoxification mechanisms are also important in natural systems, but the relative importance of each is not well known. Obviously, each mechanism will have a distinct influence on the trophic transfer of metals from primary producers to herbivores. Laboratory studies have found that a significant fraction of the metal bioaccumulated in small grazers and fish larvae can be attributed to the food pathway and that the partitioning of the metal within the algal cell largely determines the assimilation efficiency (Reinfelder and Fisher, Science 251:794, 1991). If metals are primarily accumulated intracellularly in the algal particles, these metals will be consumed by the grazer and potentially assimilated. Conversely, if the organism or phytoplankton community as a whole releases strong chelators into solution or directly excretes metal-chelate complexes then there will be less metal accumulation in the algal material and less consumed by the grazer. Thus it is critical to understand the basic metal detoxification mechanisms employed by microalgae in order to predict the fate and impact of toxic metals in an aquatic ecosystem.

FOOD WEB STRUCTURE AND HEAVY METALS IN FISH OF NORTHEASTERN U.S. LAKES

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This study assesses the influence of aquatic food web structure on bioavailability of toxic metals (e.g., As, Hg, Cd, Pb, and Zn) to humans via fish consumption. We examined metal concentrations in fish and zooplankton from a number of lakes to understand the mechanisms of metal transfer (bioaccumulation, biomagnification & biodilution) through aquatic food webs. To examine the relationship between food chain length or food web linkage and metal concentrations in fish, we selected 14 lakes which have equal chain length (= 5), but that vary in chain linkage (from < 5 to > 20) and span a range of Hg and As levels. Metals levels in different components of the zooplankton food web were measured. First, bioconcentration of both Hg and As was great in the zooplankton of all lakes. Second, there were pronounced differences in the metal levels found in different zooplankton groups. Cladocerans had higher body burdens of both Hg and As than calanoid or cyclopoid copepods. Third, there was a decrease in the ratio of Hg in larger vs. smaller zooplankton (e.g., chain magnification) as linkage increased. Changes in the relative importance of omnivores vs. herbivores, and cladocerans vs. copepods in the food web may explain this decrease. Examination of the food web appears essential to understanding mechanisms of metal transfer in aquatic systems. Differences in structural food web characteristics may explain the high variability of heavy metals among lakes and may be useful for predicting metal concentrations in fish throughout the Northeast USA.

METAL UPTAKE, DISTRIBUTION AND EXCRETION IN MAMMALS

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Heavy metals readily enter the food chain. For humans, intake in food and water, in addition to hand-to-mouth activity in children, normally accounts for the major portion of total metal in the body. Especially in the occupational environment, or as a result of smoking, inhalation exposure also can contribute significantly to body burden. Because of its primary role in metal uptake, intestinal metal absorption has been studied by a number of investigators. In the intact jejunum of mature rats, metals follow a transcellular pathway across the epithelium, but net fractional absorption is low. The process consists of discrete steps in series, and depends strongly on variables such as diet and age. Thus, for example, unidirectional cadmium efflux per unit area from the intestinal lumen of the weanling rat exceeds that in mature animals by a factor of almost three. The known properties of the individual steps will be shortly summarized. It is worth reemphasizing that temperature sensitivity of metal uptake by itself does not prove either involvement of carriers or dependence on metabolic energy. Different cell types, such as erythrocytes and excitable cells, appear to accumulate metals by steps differing from those proposed for enteric cells.

Because of their high affinity for proteins and other endogenous ligands, unbound metal ions possess only a very short half-life in the body. This was directly demonstrated in experiments where cadmium, zinc and mercuric ions became completely bound to plasma proteins within a fraction of a second following intravascular injection. Such rapid and nonspecific reactions imply that metal-induced inhibition of purified enzymes in vitro has little toxicological significance.

Metals are specifically distributed to, and accumulated in, various tissues depending on the nature of the circulating metal compound and on the properties of cell membranes and other tissue constituents. Different metals may seek out different target organs, where retention can be very long; instances are Pb in bone and Cd in renal cortex. A given metal may affect several organs, and also act at multiple sites within a cell. Hg^{++}, for instance, reacts primarily with the kidney, but also depresses function of other organs. Moreover, it inhibits renal amino acid transport at both apical and basolateral membranes. Cell barriers like the blood-brain barrier restrict distribution of different metal compounds in the body.

Absorbed metals are excreted primarily through the kidneys. Important factors here are diffusibility, filterability and dissociability of the metal compounds circulating in plasma. Tightly ligated metals such as Cd in Cd-EDTA cannot react with tubular epithelial cells, and their clearance equals the glomerular filtration rate. In contrast, Cd circulating as the cysteine complex also is freely filtered but reacts further with apical and basolateral membranes. Biliary and enteric secretion also contribute to metal excretion. However, the long biological half lives of some metals and their cumulative toxic action, make it difficult to reach a steady state.

METALLOTHIONEIN IN CYTOTOXICITY OF METALS AND APOPTOSIS

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Several metals and their compounds can cause both cytotoxicity and genotoxicity under various conditions. Some of these effects may involve mechanisms of direct damage to cell membrane, affecting certain enzymes, receptor binding or altering signal transduction pathways, and damaging certain specific cell organelles or by altering apoptosis, in addition to indirect effects such as formation of lipid peroxides, free radicals or cytokines. The induced synthesis of metallothioneins (MTs) is one of the cellular defense mechanisms in protection against metal toxicity and it may be mediated through metal binding or free radical scavenging properties of MT. The protective role of MT in cadmium toxicity has been shown in animals, cell cultures, and in transgenic MT-null mice where the two major isoforms of MT are absent. The increased drug resistance in certain tumors may depend on high expression of MT, especially the presence of MT in the nucleus. On the other hand, murine hepatocellular tumors with little MT expression can be necrotized specifically with a single, non-toxic dose of cadmium salts. The carcinogenicity of cadmium in certain tissues of animals may be related to down-regulation of MT synthesis in these tissues. Metals and other inducers of MT can modulate apoptosis, but the role of nuclear MT in this process is not yet understood. However, an inverse relation between MT expression and apoptotic bodies is observed in hepatocellular carcinoma and metastatic adenocarcinoma. It should be pointed out that the metals bound to MT can influence the protective effect against both cytotoxicity and genotoxicity. Thus, copper-MT does not protect cells but can cleave DNA at specific sites and also increase lipid peroxidation, whereas zinc-MT can be protective against toxicity. The mechanism of the toxic effects of copper-MT is most likely due to the release of a chelatable form of copper ion from copper-MT and this can participate in DNA binding or lipid peroxidation. The hepatocytes in Toxic Milk Mutant mice contain high levels of copper-MT, including in the nucleus, and they also show increased apoptotic bodies.

METALS AS ENDOCRINE DISRUPTERS

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Environmental exposure to steroid-like xenobiotics has been demonstrated to disrupt steroid hormone-mediated regulation of reproduction and development. In addition to steroid hormones, polypeptide hormones also play an important role in the regulation of these and other metabolic processes. However, little is known regarding the ability of environmental pollutants to disrupt polypeptide hormone action. The Group IIB metals (mercury, cadmium, and zinc) represent a family of environmental pollutants with the potential to disrupt polypeptide hormone function. Their toxicities, as well as their persistence in the environment, have caused mercury and cadmium to be registered as priority pollutants warranting a substantial regulatory infrastructure to control their use and release into the environment. However, one metabolic effect of these metals, which has been neglected somewhat, is their ability to specifically mimic insulin-mediated metabolism. Mercury and cadmium have been demonstrated to mimic insulin action *in vitro* whereas, zinc exhibits insulin-like and insulin-potentiating effects both in vitro and *in vivo*. The insulin-like effects of zinc are sufficiently potent to warrant consideration of its use in humans as a modulator of blood glucose levels. Some of the insulin-like effects induced by mercury, cadmium, and zinc include stimulation of RNA synthesis, stimulation of protein synthesis, and an alteration of glucose homeostasis. These modifications of glucose homeostasis occur through an increase in glucose tolerance (Zn), an increase in glucose transport (Hg, Cd, Zn) (a result of increased numbers of transporters and/or a change in transport kinetics), and an increase in glucose oxidation (Zn). All of these effects on glucose homeostasis are normally attributed to insulin and are critical in maintaining glucose homeostasis. Since mercury, cadmium, and zinc would induce these effects following environmental exposure, and not through normal physiological conditions, their activities may not be regulated by normal control mechanisms. As with the steroid-like endocrine disrupters, mercury and cadmium are persistent within the body; thus, increasing the duration of sure and the potential for cumulative actions. Indeed, long-term exposure to environmental ium has increased the incidence of renal dysfunction in Belgium. Therefore, mercury, cadmium, and zinc may represent environmental pollutants capable of exhibiting uncontrolled insulin-like effects (i.e. insulin disrupters). An inability to properly control glucose homeostasis due to an interruption in the production of or the response to insulin can culminate in dysfunctions of insulin-regulated metabolism and ultimately in the onset of diabetes. Therefore, any exogenous compound which disrupts the control of blood glucose homeostasis may contribute to the etiology this disease.

METAL-INDUCED IMPAIRMENT OF CELL VOLUME REGULATION

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Animal cells are constantly challenged with osmotic stresses created by both physiological variations in extracellular osmolarity and by the presence of high intracellular concentrations of impermeant solutes. Cells respond to these challenges by activating specific mechanisms that allow them to maintain a constant volume. Volume regulation is critical for establishing and maintaining transmembrane ion, solute, and electrical gradients that in turn regulate a multitude of cellular functions. Disruption of cell volume regulation impairs these cellular functions and may compromise survival of the cell. There is now considerable evidence that the cytotoxicity of diverse chemicals involves disturbances in cell volume regulation. Cell swelling is one of the

earliest and most conspicuous features of cytotoxicity after exposure to ethanol, carbon tetrachloride, mercury, and many other chemicals. Indeed, cell swelling and shrinkage are recognized as hallmarks of necrotic and apoptotic cell death, respectively; however, the mechanisms responsible for these changes in cell volume are not well defined. Because cell volume adjustments depend largely on the balanced functioning of membrane proteins that take up or release specific solutes, these membrane transport pathways are the most likely mediators of these effects. Early studies on the effects of mercury on renal tubule cells and erythrocytes demonstrated that mercury exposure leads to cell swelling, and that this is associated with an inhibition of ion and solute transport proteins, most notably the NaK-ATPase, and with an increase in cation permeability. Our studies in skate hepatocytes have confirmed these findings, and have identified a volume-activated osmolyte channel as an additional target of mercury-induced cell injury. We characterized a volume-activated channel in skate hepatocytes, and demonstrated that this channel is regulated by physiological concentrations of intracellular ATP, and inhibited by mercury and other sulfhydryl-reactive reagents. Our data indicate that any agent capable of altering cellular energy status will indirectly interfere in the proper functioning of the channel and with cell volume regulation. Because this channel appears to be ubiquitous, it may be a common mediator of xenobiotic-induced cell swelling.

MOLECULAR BASIS FOR EFFECTS OF CARCINOGENIC HEAVY METALS ON INDUCIBLE GENE EXPRESSION

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The heavy metals, arsenic, cadmium, chromium and nickel are each considered human carcinogens, although they are believed to act through very different mechanisms. Chromium and nickel act as classic genotoxic and mutagenic agents, and DNA/chromatin is believed to be the principal target for their effects. In contrast, arsenic and cadmium are considered non-genotoxic but are known to be able to target specific proteins, principally through sulfhydryl interactions. Recent evidence suggests that heavy metals can exert their toxic and carcinogenic effects, at least in part, through specific alterations in gene expression. We have proposed a model in which genotoxic metals such as chromium and nickel alter gene expression principally through *cis* effects on the promoters of targeted genes, whereas non-genotoxic metals such as arsenic and cadmium alter gene expression principally through *trans* effects on transcription factors and other cellular signaling pathways. We had previously shown that various genotoxic chemical carcinogens, including the metal complexes chromate and cisplatin, preferentially altered expression of several inducible genes while having little or no effect on constitutive gene expression. We were therefore interested in whether each of these carcinogenic heavy metals might target specific but distinct sites within cells, leading to alterations in gene expression which might contribute to the carcinogenic process. Arsenic and chromium, each significantly altered both basal and -hormone inducible expression of a model inducible gene, phosphoenolpyruvate carboxykinase (PEPCK) at non-overtly toxic doses in the chick embryo in vivo. We have recently developed two parallel cell culture systems to examine the basis for these effects. In the first, we are examining the effects of heavy metals on expression and activation of specific transcription factors known to be involved in regulation of inducible genes, and have recently observed significant, but different effects of arsenic and chromium. Second, we have developed a cell line with a stably integrated PEPCK reporter / luciferase reporter gene to examine effects on promoter function, and have also recently seen profound effects of these agents in this system. These model systems will allow us to identify the critical cis (DNA) and trans (protein) cellular targets of heavy metal exposure leading to alterations in expression of sensitive genes. It is anticipated that such information will provide insight into the mechanistic basis for these effects as well as provide sensitive molecular markers for human exposure.

METAL INHIBITION OF SIGNAL TRANSDUCTION PATHWAYS IN THE SHARK RECTAL GLAND

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We are pursuing the hypothesis that heavy metals exert their toxic effects on osmoregulation by interactions with specific proteins in the signal transduction pathways regulating ion transport in marine epithelial tissues. In the perfused shark rectal gland we have shown that cadmium blocks only the response of inhibitory hormones (somatostatin, NPY and adenosine) to inhibit secretagogue-stimulated secretion while actually enhancing the response to secretagogues (VIP and forskolin). In contrast, the heavy metal nickel, at low concentrations, blocks the response to secretagogues mediated via the cyclic AMP-PKA pathway but not via the cyclic GMP-PKG pathway. Specifically, we find that nickel causes: 1) a dose-dependent inhibition of VIP stimulated chloride secretion in perfused glands, with complete inhibition occurring at 10^{-5} M nickel; 2) a dose-dependent inhibition (IC_{50}, $0.8x10^{-5}$ M) of VIP stimulated tissue cyclic-AMP content in both perfused rectal glands and primary cultures of rectal gland tubules; 3) an inhibition of VIP stimulated increases in intracellular calcium, as measured by the photoprotein aequorin; 4) a lack of effect of nickel to inhibit chloride secretion stimulated by C-type natriuretic peptide (CNP); and 5) a failure of nickel to inhibit CNP stimulated tissue cyclic GMP accumulation in the perfused rectal gland. These results provide the first evidence for an effect of nickel to block selective secretagogues in a chloride secreting epithelium. Nickel at low concentrations completely inhibited VIP stimulated chloride transport and cAMP content but did not inhibit C-type natriuretic peptide stimulated chloride secretion or cGMP content. Since nickel inhibits VIP stimulated increases in intracellular calcium, the results suggest that the action of nickel may be on a calcium sensitive step in the signal transduction of VIP, possibly at a Gs protein. Nickel may be a useful tool in dissecting the crosstalk between cAMP and cGMP mediated activation of CFTR. Our present objective is to examine the molecular interactions between nickel and specific signal transduction proteins by cloning and co-expressing selective receptors, G proteins, and the CFTR-like chloride channel involved in chloride transport in the rectal gland in order to isolate the effects of heavy metals on the functional activity of these proteins.

MERCURY INHIBITION OF AMINO ACID TRANSPORT

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It is well established that mercury, in inorganic and organic forms, is capable of disrupting a wide variety of physiological and cellular activities including cell membrane transport of amino acids and other molecules. Perhaps one reason for this is that mercury forms high affinity complexes with sulfhydryl groups that are present in most cellular proteins, membrane proteins and receptors. In our studies on the uptake of the β-amino acid, taurine, by the coelomocytes (red blood cells, RBCs) of the marine polychaete, *Glycera dibranchiata*, we have shown that mercuric chloride readily inhibits taurine transport (50% inhibition for a 1 min exposure at a concentration of 20 μM). The taurine uptake system is specific for β-amino acids, Na-independent and has a Kt for taurine of about 1 mM. Mercurial inhibition of taurine transport is rapidly reversible by exposure of mercuric chloride-treated cells to low molecular weight sulfhydryl reducing agents such as dithiothreitol (DTT) and penicillamine (10 mM for 10 min). Larger molecular weight reducing agents such as glutathione have little or no capability to reverse inhibition, suggesting that the moieties that mercury reacts with may be occluded to some extent by the cell membrane.

Comparative studies using *p*-chloromercuriphenyl sulfonic acid (PCMBS) show that this mercurial reacts more slowly (50% inhibition for a 1 min exposure at a concentration of 1 mM), although this inhibition was also reversible with DTT. We also showed that mercuric chloride inhibition of taurine transport cannot be attributed to indirect effects since under the experimental conditions used for these experiments there are no changes in Na/K ion gradients, ^{86}Rb efflux rates, cell volume or transmembrane potential. In experiments in which the chloride in the incubation medium (artificial seawater) was replaced with gluconate or other anions, it was found that mercury inhibition depended on chloride concentration, reaching a maximum effect at about 100 mM. This is consistent with the possibility that the reactive form of mercury with this transport system is $HgCl_3^-$, the form that reaches its highest concentration at about 100 mM chloride. Addition of low concentrations Br^- or I^- to the medium partially prevents $HgCl_3^-$ inhibition of transport, in a manner that is consistent with formation of higher affinity complexes of these anions with Hg, resulting in formation of less reactive species. We have also shown that quinidine, which blocks anion channels under certain conditions in other cells, irreversibly inhibits taurine transport in *Glycera* RBCs. Furthermore, it appears that the degree of inhibition of quinidine and mercuric chloride when used on the same cells depends to some extent on the order of treatment, with less inhibition occurring when quinidine is added first. These experiments suggest that quinidine may block access of $HgCl_3^-$ to the transport system. These data are consistent with the possibility that the taurine uptake system in *Glycera* RBCs has some characteristics that resemble an anion channel or an anion selective transport system. We are continuing studies using molecular techniques to probe in more depth the targets of mercury interaction with this system. These data also suggest that similar approaches may be useful in characterizing molecular interaction of mercury with other transport systems.

INHIBITION OF PLASMA MEMBRANE COTRANSPORTERS BY HEAVY METALS

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Epithelia that cover the inner and outer surfaces of the body are frequently the first barrier at which heavy metals, such as mercury and cadmium, interact with aquatic and nonaquatic organisms. Such interactions result in impairment of transepithelial nutrient, salt, and water transport that are critically involved in maintaining the milieu interieur of the body. The action of heavy metals on these transport processes has been elucidated in recent years at the cellular, subcellular, and molecular level. The primary active Na,K-ATPase that maintains the driving forces for secondary active sodium-solute cotransport systems has been demonstrated to be inhibited by mercury and cadmium, thereby sodium cotransport is indirectly inhibited. The site of interaction includes the magnesium binding site facing the cytoplasm or the potassium binding site, which is accessible from the cell exterior. Sodium cotransport systems are also direct targets for heavy metals, thus sodium-proline, sodium-alanine, and sodium-glutamate cotransporters are inhibited in their activity at the cytoplasmic face of their catalytic protein unit. In contrast, the Na-K-2Cl cotransporter interacts with mercury at its extracellular face. The cloning of transporters, the determination of their amino acid sequence and of their membrane topology now makes it possible to exactly determine the side and the site(s) of interaction of heavy metals with transporters, thereby refining our knowledge both about the mechanism of action of environmental pollutants and about the structure-function relation of sodium cotransport systems.

THE SIXTEENTH ANNUAL WILLIAM B. KINTER MEMORIAL LECTURE

METHYLMERCURY TOXICOLOGY

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True to its name as "Messenger of the Gods", mercury is the most mobile element in the periodic table, physically, chemically and environmentally. Released to the atmosphere from both natural (volcanoes) and anthropogenic sources (incinerators, coal burning power stations), the monatomic vapor (Hg^0) distributes globally before returning in rain water after oxidation to the divalent species (Hg^{++}). The latter is subject to methylation by microorganisms present in sediments of bodies of fresh and ocean water.

Methylmercury compounds are bioaccumulated up the aquatic food chain reaching the highest concentrations in the tissues of predatory, long lived fish and certain species of marine mammals. The aquatic food chain is the predominant if not only source of human exposure to methylmercury. The public health challenge today is to balance the putative health risks from methylmercury, versus the beneficial aspects of fish consumption.

Several mass outbreaks of methylmercury poisoning along with extensive experimental studies have revealed the main features of methylmercury toxicology. In adults, damage is almost exclusive to the central nervous system and is associated with specific areas of the brain. Following a latent period of one or more months, the neurological signs and symptoms first manifest themselves as paresthesia followed by more serious effects such as ataxia and constriction of the visual fields. The damage is irreversible as the nerve cells are destroyed.

The prenatal period is believed to be the most susceptible segment of the life cycle. Cell division and neuronal migration are the processes primarily affected, probably through the tory action of methylmercury on the assembly of microtubules. At high doses, infants suffer severe brain damage manifested as cerebral palsy. At lower levels, prenatally exposed children fail to meet normal developmental milestones and exhibit subtle neurological and psychological deficits. To date, no clinical cases either in adults or in prenatally exposed children have resulted from consumption of methylmercury naturally methylated and bioaccumulated in fish. The results of epidemiological investigations looking for subtle effects of methylmercury will be compared with current human exposures.

THE SECOND ANNUAL MARINE BIOTECHNOLOGY SYMPOSIUM "COMMERCIAL ASPECTS OF MARINE BIOTECHNOLOGY"

Thomas Veach Long, II, Ph.D.
Maine Center for Innovation in Biotechnology
75 Market Street, Suite 305
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The Symposium was opened with welcoming statements by Barbara Kent, Ph.D., Administrative Director of MDIBL and Thomas Veach Long, II, Ph.D., C.E.O. of the Maine Center for Innovation in Biotechnology. The kick-off speaker was Oskar R. Zaborsky, Ph.D., of the University of Hawaii, whose subject was "Marine Biotechnology: The Time Has Come." Dr. Zaborsky is Director of the UH Manoa Marine Biotechnology Center and Williamson-Matsunaga FREE Scholar in Hydrogen Systems at the Hawaii Natural Energy Institute. Elaborating on his theme, he provided a comprehensive overview of marine biotechnology and its commercial possibilities. Specific technology targets are energy production by marine photosynthetic microbes, including hydrogen production by microalgae; detection of noxious residuals in the environment and bioremediation by marine organisms; and the production and extraction of valuable natural products from marine organisms, including carotenoids, fatty acids, pharmaceuticals and enzymes. Marine bioproducts are projected to be the basis for development of a multibillion-dollar industry over the next several decades. Marine biotechnology activities in specific geographic locales were highlighted, with a focus on the vigorous activity in Japan and the Pacific Rim. Finally, Professor Zaborsky discussed a proposed Marine Bioproducts Engineering Center (MarBEC). This is a multi-disciplinary engineering-basic science cooperative effort of the UH Manoa and the University of California at Berkeley. The proposed center will build the foundations of engineering research for a 21st-century marine bioproducts industry that will span the chemical, pharmaceutical, nutraceutical, cosmetics, food, and feed-ingredient sectors.

Noriko Kusukawa, Ph.D., Technical Director of FMC BioProducts, presented recent advances in her company's efforts to expand "Agarose Utilization in Molecular Biology Research." FMC BioProducts is located in Rockland, Maine, and is one of the largest firms in the world based primarily on the utilization of materials derived from marine organisms. Originally, the company extracted carrageenan from seaweeds and was known as Marine Colloids. Since 1990, it has concentrated on meeting the needs of the advancing DNA-separations technology. Agaroses that are ideal for separating PCR products and for pulsed-field electrophoresis have been added to their palette of products. Recently, they have expanded into matrices based on polyacrylamide for electrophoretic applications, ensuring continued leadership in this important area.

"Natural Products from Marine Microorganisms" was the subject chosen by Trevor Castor, Ph.D., President and C.E.O. of Aphios Corporation of Woburn, Massachusetts. Dr. Castor describes Aphios as a "green" biopharmaceutical company, which utilizes renewable terrestrial biomass and marine microorganisms, state-of-the-art micro- and molecular biology techniques, and environmentally-benign, supercritical-fluid technologies. Their concentration is on drugs for cancer, AIDS and other life-threatening diseases. Aphios' proprietary supercritical

fluids technology ("*SuperFluids*™") is utilized to enhance the discovery, manufacture, delivery, and safety of natural therapeutics derived from marine microorganisms. The microbes are collected from environments that range from mangrove swamps to deep-water habitats. Bristol-Myers Squibb is screening Aphios' library of small marine molecules for physiological activity and specificity. *SuperFluids*™ has also been used to develop stable and effective liposomal formulations of paclitaxel and camptothecin, potent anti-cancer agents.

Bruce Sidell, Ph.D., spoke on his elegant research on Antarctic fishes: "Myoglobin Expression in Antarctic Fishes: Now You See It; Now You Don't." Professor Sidell is Director of the new School of Marine Sciences at the University of Maine, Orono. The School of Marine Science is projected to be the focal point for the reinvigoration of Maine's marine industry. Plans include a major initiative in marine cold-water research. Dr. Sidell's two enduring areas of research interest are comparative and evolutionary aspects of cardiac metabolism in vertebrate animals and physiological and biochemical adaptations necessary for maintenance of normal cellular function at cold body temperature. He has determined that some icefish are able to survive and transport oxygen without either hemoglobin or myoglobin, which are normally present in vertebrates. In others, he has correlated myoglobin expression with capacities for aerobic energy metabolism among Antarctic species' heart ventricles. Although he does not speculate on possible applications, this research has seeming implications for new insights into improved cardiac function and efficient use and transport of oxygen.

Following lunch, Christopher Pazoles, Ph.D., Vice President for Research of Phytera, Inc., summarized that company's directions in "Marine Microorganisms in Drug Discovery." Phytera is located in Worcester, Massachusetts, and has developed proprietary techniques for natural product lead-structure identification: ExPand™, a plant-cell culture technology, and μMARINE™, a marine microbial culture program. These technologies are allied with high-throughput screening to identify infectious disease drug candidates. Phytera has developed a leadership position in hepatitis C and drug-resistant bacterial and fungal diseases. Once candidate structures are identified, they are optimized with combinatorial chemistry techniques obtained in the company's acquisition of Auda Pharmaceuticals, ApS. Other acquisitions include Plant Science Limited and Neptune Pharmaceuticals, Inc. The firm's product portfolio includes Marinovir for herpes infections and Sunillin, its lead antifungal.

The research program at the Bigelow Laboratory for Ocean Sciences in Boothbay Harbor, Maine, was explored by Louis E. (Sandy) Sage, Ph.D., C.E.O., and Robert Anderson, Ph.D., Director of the Provasoli-Guillard Center for the Culture of Marine Phytoplankton. Their topic was "Developing Commercial Possibilities from the National Phytoplankton Collection." While Bigelow's research has concentrated on critical global issues involving oceanographic applications of satellite imagery, primary productivity of the world's oceans, and fisheries ecology, their attention has turned increasingly to developing profitable applications of marine phytoplankton and zooplankton in their collection. The Provasoli-Guillard Center for the Culture of Marine Phytoplankton was established with funds from the National Science Foundation, Friends of the Bigelow Laboratory, and the New York Community Trust as a living archive of marine microalgae and represents one of the largest collections in the world. Bigelow currently is developing relationships with major pharmaceutical and chemical companies to

determine possible applications and large-scale production methods for organisms in its collection.

The evolution of a new venture within the confines of MDIBL was proudly related by H. William Harris, M.D., Ph.D., of Harvard University and C.E.O. of AquaBio Product Sciences: "APS: a Marine Biotechnology Start-up Based on Proprietary Calcium Receptor Technology." APS's technology platform derives from research by Dr. Harris and his colleagues Drs. Edward M. Brown of Harvard University and Steve C. Hebert at the Vanderbilt University School of Medicine. The team's calcium receptor technology was licensed to NPS Pharmaceuticals of Salt Lake City, Utah, which is exploiting human applications, while APS has rights to marine organism uses. APS and NPS have entered into an agreement for sharing research results, where appropriate. APS is concentrating on three applications: controlling cultured pearl growth to produce more perfect and larger pearls, intervening in the molting process that produces soft-shell crabs, and improving the yield of lumpfish caviar.

Finally, Thomas Veach Long, II, Ph.D., in his capacity as Chairman of Maricultura, Incorporated, a Wrightsville Beach, North Carolina marine biotechnology firm, related the development of that company's marine lipid products. Dr. Long founded Maricultura, which is one of the earliest marine biotechnology ventures, in 1984. Its proprietary technologies include the production of carotenoids, such as astaxanthin and beta-carotene, based on the fermentation of heterotrophic marine microbes. Astaxanthin is incorporated in feeds for aquacultured salmon and shrimp to provide desired pigmentation. It sells for $2,600 per kilogram and has a worldwide market estimated to approach $200 million. Maricultura also developed fermentation technology for omega-3 and other highly polyunsaturated fatty acids. A Fortune 50 company acquired its technologies in 1995 and is responsible for commercialization.

The Symposium was sponsored by MDIBL and the Maine Center for Innovation in Biotechnology.

AQUEOUS HUMOR FORMATION AND THE DEVELOPMENT OF NEW CARBONIC ANHYDRASE INHIBITORS

A Symposium Honoring Thomas A. Maren, Ph.D.
Dahlgren Hall
Mount Desert Island Biological Laboratory
Salsbury Cove, Maine
June 27, 1997

Henry F. Edelhauser
Director of Ophthalmic Research, Emory Eye Center
1365B Clifton Road, NE, Suite B2600, Atlanta, GA 30322

This symposium was held to honor the many contributions of Dr. Thomas A. Maren in aqueous humor formation and understanding the basic biology of ciliary body which lead to the development of a topical drug to lower intraocular pressure in glaucoma patients. The symposium was sponsored by Merck and Company. The attendees of the symposium were longtime colleagues and former fellows and students of Dr. Maren. They reviewed some of the studies pertaining to aqueous humor formation and glaucoma and paid tribute to Dr. Maren.

Dr. Maren was the first speaker at the symposium. He presented an historical account of the development of carbonic anhydrase inhibitors and their use in the eye. He was followed by Paul L. Kaufman, M.D., Professor of Ophthalmology from the University of Wisconsin who spoke on the effects of novel compounds and fluorophotometry in measuring aqueous humor flow in monkeys. Kristine A. Erickson, Ph.D., Department of Ophthalmology at Boston University summarized her studies on the physiology of aqueous humor dynamics. A longtime friend of Dr. Maren, Marvin L. Sears, M.D., Emeritus Professor and past Chairman of the Department of Ophthalmology at Yale University spoke on homologous desensitization of the Beta adrenergic receptor and circadian rhythm of aqueous flow. Thomas W. Mittag, Ph.D., Professor of Ophthalmology and Pharmacology at the Mount Sinai School of Medicine summarized the role of bicarbonate and cyclic AMP in aqueous humor formation. David L. Epstein, M.D., Chairman of Ophthalmology at Duke University presented a wonderful tribute to Dr. Maren for all of the contributions that he has made to our understanding of aqueous flow and also provided a summary of the mechanisms of aqueous outflow through the trabecular meshwork. Jose A. Zadunaisky, M.D., Ph.D., Professor of Ophthalmology at the University of Miami and a principal investigator at MDIBL spoke on the active role of the trabecular meshwork cells in the control of intraocular pressure. He was followed Theodore Krupin, M.D., Professor of Ophthalmology at Northwestern University, who spoke on his studies on trifluoromethozolamide and the potential of further, new carbonic anhydrase inhibitors to lower intraocular pressure. The last presentation of the morning session was given by Albert R. Frederick, M.D., a retinal surgeon from the Ophthalmic Consultants of Boston. Dr. Frederick was the first medical student that Dr. Maren invited to MDIBL from the University of Florida. Dr. Frederick recalled his early relationship as a young scientist and clinician with Dr. Maren.

The afternoon session was devoted more to the specific information as to how topical drugs lower intraocular pressure. Michael F. Sugrue, Ph.D., from Merck and Company, spoke on Dorzolamide and it's pharmacological properties and reviewed many of the basic and clinical studies in the development of this drug. He was followed by Robert L. Smith, Ph.D., a retired scientist from Merck and Company. It was Dr. Smith that brought Dr. Maren's observation from the laboratory that topical carbonic anhydrase inhibitors could become a reality and it was Dr. Smith who, along with the other Merck scientists, brought the topical carbonic anhydrase inhibitors to a reality as a clinical product. Henry F. Edelhauser, Ph.D., Director of Ophthalmic Research at Emory University summarized the initial studies he conducted in collaboration with Dr. Maren on cornea and scleral permeability in the development of topical carbonic anhydrase inhibitors. Curtis W. Conroy, Ph.D., from the University of Florida, summarized his more recent studies on cornea versus scleral penetration of Methazolamide in the ionized state. Jonathan H. Lass, M.D., Professor and Chairman of the Department of Ophthalmology at Case Western Reserve University summarized the clinical studies on the corneal safety of Dorzolamide, Timolol and Betaxolol after one year of therapy. Amir Bar-Ilan, Ph.D., a senior scientist at Pharmos Corporation in Israel and a long-term colleague of Dr. Maren, spoke on aqueous humor flow changes following topical carbonic anhydrase inhibition in rabbits. Nicholas A. Delamere, Ph.D., Professor of Ophthalmology at the University of Louisville, spoke on Acetazolamide induced changes in aqueous composition - clues to the cellular effects of carbonic anhydrase inhibitors on ion transport. He was followed by Maher M. Fanous, M.D., Professor of Ophthalmology at the University of Pittsburgh, who spoke on the effect of topical versus systemic carbonic anhydrase inhibitors in rabbits. Beth R. Friedland, M.D., from the Department of Ophthalmology at the University of North Carolina and also a former MDIBL research fellow with Dr. Maren, spoke on the clinical trials and patient experience with topical carbonic anhydrase inhibitors. The last speaker of the day was Thom J. Zimmerman, M.D., Ph.D., Professor and Chairman of Ophthalmology and Visual Science at the University of Louisville. Dr. Zimmerman spoke on the safety of topical carbonic anhydrase inhibitors: an important addition to lower to intraocular pressure.

All of the speakers expressed their extreme gratitude and complemented Dr. Maren for his many years of hard work to bring topical carbonic anhydrase inhibitors to reality. This new drug "Dorzolamide" is one of the first new drugs on the market for lowering intraocular pressure in glaucoma patients. This drug has been shown to be safe and effective and has provided the ophthalmologists with an important new drug.

The symposium ended with a banquet at the Bayview Inn in Bar Harbor. All the participants and attendees were very grateful for the opportunity to come to the MDIBL and pay tribute to Dr. Thomas A. Maren. (This symposium was sponsored by Merck and Company.)

REPORT TITLES