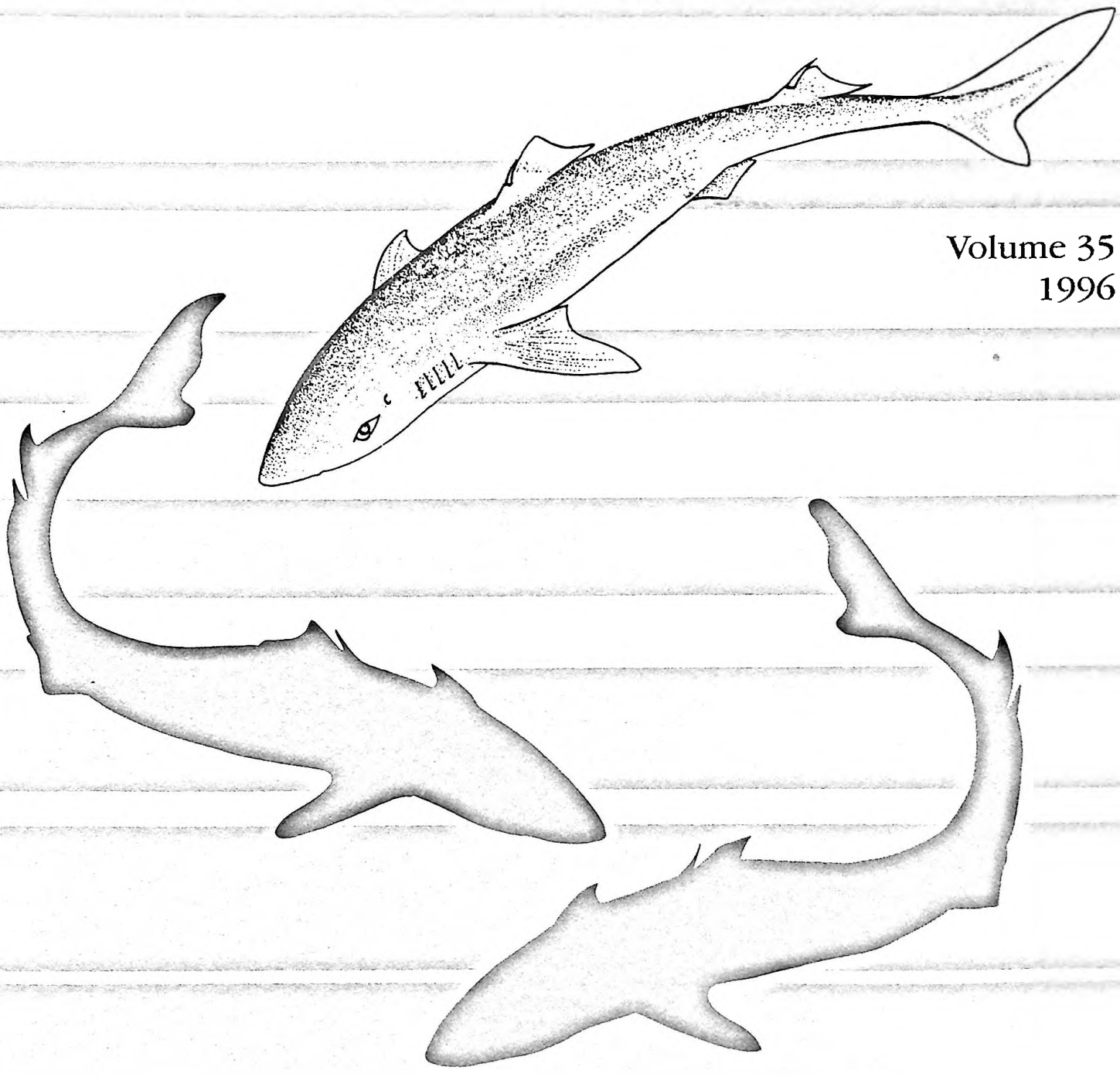
THE BULLETIN

Mount Desert Island Biological Laboratory



Volume 35
1996

Printed on Recycled Paper

Published by the MDI Biological Laboratory
March 1996
$10.00

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Volume 35 1996

Mount Desert Island
Biological Laboratory
Salsbury Cove, Maine 04672

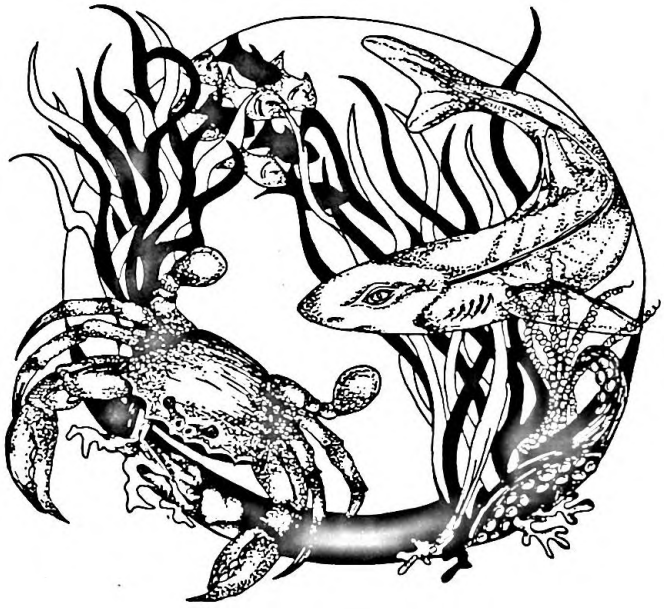

TABLE OF CONTENTS

Introduction ..... ii-iii
The Homer W. Smith Symposium 1995:
Reflections and Impressions, Rolf
K. H. Kinne and E. Kinne-Saffran ..... iv-xv
Report Titles ..... xvi-xix
Reports 1-111
Officers and Trustees 112-114
Scientific Personnel 115-119
Seminars 120-122
Publications 123-124
Author Index 125-126
Keyword Index 126
Species Index 127-128

THE MOUNT DESERT ISLAND BIOLOGICAL LABORATORY

SERVING HUMANITY THROUGH
RESEARCH AND TEACHING IN MARINE BIOMEDICINE

INTRODUCTION

The Mount Desert Island Biological Laboratory (MDIBL) is an independent non-profit biological station. It is 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 1995, the scientific personnel included 49 principal investigators, 70 associates and 11 assistants/technicians, representing 51 institutions in 25 states and 5 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., 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 biomedical research and teaching 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 434 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 the general administration and long range goals of the Laboratory. The Director, with the aid of a full-time Administrative Director and staff, 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), is the use of marine animals like the shark, the flounder 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 wide-spread 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 & 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. Applications 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 Burroughs Wellcome Fund, Grass Foundation, Milbury Fellowship Fund, American Heart Association - Maine Affiliate, Mr. Robert E. Blum, Maine Community Foundation, NSF - Research Experience for Undergraduates and NSF Young Scholar Program for High School students and many local businesses and individuals.

THE HOMER W. SMITH SYMPOSIUM 1995: REFLECTIONS AND IMPRESSIONS

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Preface

On the occasion of the 100th anniversary of his birth an international symposium honouring Homer W. Smith was held on Mount Desert Island under the auspices of the Mount Desert Island Biological Laboratory from August 15 till August 19, 1995. The idea to organize such a symposium originated from Yuri Natochin, who discussed it with Klaus Beyenbach, who in turn got the Kinne's and the MDIBL involved. The stone that had been thrown in the water created ever growing circles of interest that soon reached Barry Brenner, who added the aspects of clinical nephrology to the program and secured the funding of the symposium.

But how does one honour appropriately such an eminent man, who excelled as "Scientist, Teacher, Explorer, Novelist, and Perennial Student" [S.J. Farber, Kidney Int. 49, 1996]. As scientists the organizers decided to dedicate most of the symposium to evaluate the progress made in renal physiology and clinical nephrology since Homer W. Smith deceased in 1962. As a guideline we selected his pivotal monograph: The Kidney: Structure and Function in Health and Disease, published in 1951 [Oxford University Press, New York] and used its title for the symposium.

Honouring Homer W. Smith meant not only to recall his role in nephrology "a field that he dominated for over thirty years in a way few (if any) have dominated other fields" [R.F. Pitts, National Acad. Sci. 39, 445-470, 1967], but also to pay tribute to some of the other prominent aspects of Smith's multifacetted personality and interests. One of these was his influence on others: "In fact, I believe it would be fair to say, that Smith's contributions to renal physiology and clinical nephrology should be measured even more by his influence on others and their productivity than by his own scientific achievements, great as they were. A generation of renal physiologists and clinical nephrologists owe their expertise to the direct influence of Homer W. Smith" [D.S. Baldwin, Kidney Int. 49, 1996]. Such influence did manifest itself in several ways. For many participants at the symposium his 1937 book on the Physiology of the Kidney [Oxford University Press, New York] was their fist significant exposure to the subject, "and provided the real impetus for them to have a try at that field" because observations of various origins had been synthesized into a "logical and consistent picture of how the kidney works" [R.W. Berliner, Kidney Int. 49, 1996]. Homer W. Smith had also a deep

interest in "mentorship at various levels of sophistication... and a very special concern for and guidance of his mentees" [I.L. Schwartz, Kidney Int. 49, 1996]. This tutelage reached from individual mentor-mentee relationships to medical education in general.

Therefore, in order to get (and provide the participants of the symposium with) a glance of Homer W. Smith as a human being, at the opening event disciples and acquaintances of Homer W. Smith were asked to share their experiences and impressions and set the tone of the symposium to come.

In addition, a Round Table Discussion was incorporated into the symposium, entitled 'Nephrology in 1995: More and More Details, Less and Less Synthesis. Is this the Smith Legacy?', in which the current concerns about a proper balance between the reductionistic and holistic approach to renal physiology and medical education was debated.

A third area of Smith's interest was comparative physiology and evolution. To cover this topic a special lecture was arranged, and we were lucky to recruit Stephen Jay Gould as a speaker.

And last, but not least, Smith's instrumental role in the scientific life of the MDIBL was highlighted by an exhibit 'Homer W. Smith at Work at the MDIBL', arranged by Carl W. Gottschalk.

If one leans back and asks oneself what do you remember most of the symposium it is ... the weather. We had a perfect week in August with bright sunshine, which bathed the short commute, the coffee breaks and the receptions in brilliance and beauty. And we had the breeze, moving the shades in the auditorium from time to time as if to remind us of the world outside the ivory tower we were contemplating in, and like in a sail boat filling the sails of science to reach new frontiers. Then the participants come to mind - in their cheerful mood they created the spirit of the symposium and lived it. Some of them had not seen each other for years, others - especially the more junior scientists - met for the first time men and women who shaped the field of nephrology for the last decades. And of course the scientific presentations, thoughtfully prepared and masterfully presented.

In the following we will share with you our reflections on the outcome of this endeavor and highlight some of the recent developments in those scientific areas Homer W. Smith was and investigators at MDIBL currently are interested in. The account is very personal but we think that we can afford this privilege

because the Proceedings of the Symposium will be published in a forthcoming issue of Kidney International this summer[1].

Excretion of Urea*

Until recently, our thinking about the handling of urea by cells and in the renal medulla was governed by the assumption that urea easily crosses cell membranes by simple lipid diffusion and that its movements are governed by passive transport across the epithelia. Both assumptions have been challenged in recent years. Sands et al. [ibid] provided clear evidence that in rats, when fed a low protein diet, the initial inner medullary collecting duct, when perfused in vitro, transports urea actively. Since removal of sodium from the tubular lumen or addition of ouabain to the basal-lateral cell side reduces transport significantly, it is postulated that the transport is secondary active involving a sodium-urea cotransport system in the luminal membrane.

As reviewed by Hediger et al. erythrocytes and epithelial cells of certain nephron segments have urea permeabilities that are considerably higher than expected from simple diffusion, suggesting the presence of special urea transporters. Some of these transporters have now been cloned and identified at the molecular level. Expression cloning from rabbit and subsequently from rat kidney has led to the identification of a novel phloretin-sensitive urea transporter, called UT2. The sequence predicts a 43 kDa polypeptide with ten transmembrane domains and one predominant extracellular loop between transmembrane helix 5 and 6. Rat UT2 has two transcripts in the kidney, one of 2.9 kb and another of 4.0 kb that show a different distribution in the kidney. The 2.9 transcript is found in highest concentrations in the inner stripe of the outer medulla and the inner medulla, whereas the 4.0 kb transcript is present mainly in the inner medulla and papilla of the rat. In addition, the two transcripts respond differently to various physiological states of the animals. The 4.0 transcript is regulated by the dietary protein uptake and the 2.9 kb transcript by the hydration state of the animal. Thus, tools are now available to further characterize the molecular and cellular events underlying the regulation of urea transport in the medulla. The information at the molecular level will undoubtedly also help to further clarify the complex pathways and mechanisms of urea recycling that have been brought into focus again by Bankir et al. [ibid].

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[1] All articles cited in the following will be published in Kidney International, Vol. 49, 1996 as Proceedings of the Homer W. Smith Symposium

* Indicates a title of a chapter in The Kidney: Structure and Function in Health and Disease

Clearances Involving Active Tubular Reabsorption*

The field of tubular transport is currently dominated by two major themes: the molecular identification of the pumps, channels and transporters involved in tubular transport and the cellular events regulating the activity of transport systems either in loco or by sorting to the appropriate membrane domains. Establishment and maintenance of the biochemically, structurally and physiologically distinct apical and basolateral domains of the plasma membranes of epithelial cells thereby is of central importance for the proper function of these cells. As demonstrated by Molitoris and Wagner alterations in this process can be the basis for malfunction. Ischemia, via intracellular ATP-depletion, leads for example to a loss of proximal tubule cell surface membrane polarity. Of major importance for this process to take place is a rapidly occurring, duration-dependent disruption and dissociation of the actin cytoskeleton and associated surface membrane structures. This results in loss of cell-cell contact, cell-extracellular matrix adhesion and surface membrane polarity. A distribution of surface membrane proteins and lipids into alternate domains of the plasma membrane ensues with severe impairment of the vectorial transepithelial transport. These changes are reversible and the repair is probably supported by growth factors. The extracellular matrix has also been shown to be relevant, in addition to soluble mediator substances, in determining the proliferative and synthetic type of the glomerular mesangial cells. Changes in the extracellular matrix therefore may have a pivotal role in the altered behavior of mesangial cells in glomerular diseases of the kidney [Ruprecht et al.; Schlöndorff, ibid].

Clearances Involving Tubular Excretion*

The demonstration of tubular secretion in the kidney is strongly associated with E.K. Marshall, the MDIBL and the power of comparative physiology. With regard to the cellular mechanisms involved in the active secretion of organic anions Pritchard and Miller reported on a novel mechanism. According to their recent findings a significant fraction of organic anions having entered the cell via the dicarboxylate-organic anion exchanger is sequestered into vesicles. Disruption of the cellular microtubular network can lead to both, diminished vesicular movement within the cell and reduced transepithelial transport. Thus, vesicular transport appears to play a much more significant role in organic anion secretion than previously assumed. Whether the substantial intracellular sequestration of organic cations is also related to transepithelial secretion remains to be determined.

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* Indicates a title of a chapter in The Kidney: Structure and Function in Health and Disease

The Antidiuretic Hormone and the Excretion of Water*

The understanding of the action of antidiuretic hormone on the water permeability in the collecting duct and the basis for the high hydraulic conductivities in the proximal tubule, the thin descending limb of Henle's loop and the collecting duct (in the presence of ADH) has increased steadily over the last decades and has now reached the molecular level. The new tools of cell biology and molecular biology do not only allow to describe in more detail the physiological events of water transfer across epithelia and its regulation by ADH but also to gain insight into the molecular basis of nephrogenic diabetes insipidus.

As reviewed by Knepper et al. and Nielsen et al. [ibid] the high hydraulic conductivity of certain tubular structures can be explained by the presence of water channels or 'aquaporins' in the plasma membranes of these cells. There are several aquaporins that differ in their regional distribution, cellular location and regulatory response. AQP1 is constitutively expressed at very high levels in the proximal tubule and in the descending limb of Henle's loop and present in both the apical and basal-lateral membranes of the cells, suggesting that rapid water transport across these epithelia is mediated by this water channel at both cell sides. AQP2 is found predominantly in the apical plasma membrane and in subapical membrane vesicles within principal cells of the collecting duct in cortex, outer medulla, and inner medulla. AQP2 is also the 'vasopressin-regulated water channel' that, as response to a stimulus by ADH, is incorporated into the luminal membrane by the 'shuttle mechanism'. These vesicles seem to be guided to the plasma membrane by 'vesicle-associated membrane proteins' such as synaptobrevins and by target membrane-associated proteins, such as syntaxin as demonstrated also by Hays [ibid]. Thus, the principles of cytoskeletal control and vesicle docking found in nerve terminals or the chromaffin cell seem to apply also to the collecting duct cells. AQP2 is also the target of long-term regulation of water permeability of inner medullary collecting duct (IMCD) cells. For example, restriction of fluid intake in rats for 24 hours leads to a marked increase of AQP2 expression in the apical membrane as well as the subapical membrane vesicles. Concomitantly, the corresponding mRNA was found to be augmented. AQP3 and AQP4 are present in the basolateral membranes of collecting duct principal cells and IMCD cells, but not elsewhere in the kidney. AQP3 is dominant in the lateral plasma membrane, whereas AQP4 is distributed almost equally between the basal and the lateral membranes. AQP2 mutations have been found in humans with primary nephrogenic diabetes insipidus and the lack of appropriate expression of AQP2 has been invoked in acquired forms of nephrogenic diabetes insipidus from a variety of causes.

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* Indicates a title of a chapter in The Kidney: Structure and Function in Health and Disease

Vasopressin receptors that initiate the chain of events comprising the action of ADH on the water permeability of the collecting duct have also been cloned recently and have been studied in great detail with regard to their role in nephrogenic diabetes insipidus. As reviewed by Bichet, the human V2 receptor gene is located in chromosome region Xq28 and has three exons and two small introns. The cDNA sequence predicts a polypeptide of 371 amino acids with a structure typical for G-protein-coupled receptors with seven transmembrane, four extracellular and four cytoplasmic domains. As reported by Bichet [ibid], 68 putative disease-causing mutations in the gene of the human V2 receptor have now been reported in 95 presumably unrelated families with x-linked nephrogenic diabetes insipidus. These mutations in most cases can lead to one of three phenotypes: (1) impairment of hormone binding to the receptor at the cell surface, (2) blocked intracellular transport of the receptor, or (3) ineffective biosynthesis and/or accelerated degradation of the receptor. These studies are prime examples "to allay the concern ... that renal physiologists have abandonned the sick to pursue complicated and specious theorems in ivory towers, or have overlooked ... that in this molecular world, structure always underlies function [H.W. Smith, The Kidney: Structure and Function in Health and Disease].

Excretion of Sodium and Other Strong Electrolytes*

Homer Smith in his 1937 book [Physiology of the Kidney] wrote: "The history of renal physiology has erred, more often than not, by attempts at oversimplification. The problems of water and salt excretion appear to be extremely complex, and especially liable to this danger". The papers presented at the symposium confirm this complexity. Blaustein discussed the problem how impairment of sodium excretion - the major culprit in the pathogenesis of hypertension - leads to increased vascular resistance and elevation of the blood pressure. He invoked a recently discovered adrenal cortical hormone, 'endogenous oubain', as the mediator. This hormone inhibits the sodium pump and raises intracellular sodium in many cells. Due to a decrease in Na/Ca exchange the intracellular calcium and, more importantly, the capacity of intracellular calcium stores are increased. Consequently, the vascular smooth muscles, vasomotor neurons, and endothelial cells become hypersensitive to activation. This may account for the increased arterial tone and peripheral vascular resistance. Aperia et al. also proposed that the Na,K-ATPase is a major site of regulation for sodium balance. Her group demonstrated that the Na,K-ATPase protein is subject to an intricate system of phosphorylation (leading to inhibition) and dephosphorylation (leading to reactivation). These

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reactions are mastered by hormones such as dopamine and norepinephrine, which exert opposing forces on a common intracellular signalling system. This model also applies to the action of the natriuretic factor and its second messenger cGMP as well as to the antidiuretic factors, angiotensin and neuropeptide Y.

Na,K-ATPase has been the first sodium transport system to be identified in molecular terms and thus could be studied extensively with regard to its role in sodium homeostasis. Other sodium transport systems have followed suit recently. Aronson reported on the identification of the molecular entity of the Na/H exchanger in the proximal tubule that is responsible - in conjunction with the chloride-formate and chloride-oxalate exchangers - for the bulk of both, $NaHCO_3$ and NaCl reabsorption. There are several isoforms of Na/H exchangers that have been cloned. Out of these NHE1 appears to be located only in the basal-lateral plasma membranes of renal cells and is probably mainly involved in the regulation of intracellular pH. NHE3, on the other hand, in rat and rabbit kidney is predominantly localized in the brush border membrane of the proximal tubule as well as in subapical membrane vesicles. Apical membranes of other renal cells show only weak staining for NHE3. From functional studies the author concludes that "virtually all Na/H exchange activity in the renal brush border membranes is mediated by NHE3 under baseline conditions and during upregulation ... associated with metabolic acidosis, renal maturation, and glucocorticoid administration".

Also the major sodium transporter in the thick ascending limb and the distal tubule have now been identified. As reviewed by Hebert et al. the Na-K-2Cl cotransporter in the luminal membrane of the thick ascending limb of Henle's loop and the NaCl cotransporter in the early distal tubule evolved from a common ancestral gene and form a new gene family. The electroneutral sodium-chloride transport mechanism was first cloned from flounder urinary bladder, one of the model systems extensively used at the MDIBL, and isoforms have been found in a variety of mammalian species. Due to its sensitivity to thiazides it is called TSC (thiazide-sensitive sodium-chloride transporter). Bumetanide-sensitive Na-K-2Cl cotransporters, termed BSC, were identified thereafter. In the mouse BSC1 and BSC2 are the products of different genes and also exhibit different cellular locations and cellular function. BSC1 seems to be responsible for sodium-chloride reabsorption via secondary active chloride transport in the thick ascending limb of Henle's loop. BSC2 mediates Na-K-2Cl cotransport in secretory epithelia such as the rectal gland of the shark and is involved in volume regulation during cell shrinkage in a variety of tissues.

Finally, Benos et al. summarized the current status of knowledge on amiloride-sensitive sodium channels that modulate to a fine degree the final composition of the urine and thus are essential for sodium homeostasis. The unique feature of these

channels is the wide variety of regulatory signals to which they respond. The activity of the channels is regulated by vasopressin, aldosterone, insulin, and atrial natriuretic factor. In some cases these hormonal effects are not only additive, but synergistic. Potential intracellular modulators, that have been identified very clearly in experiments employing purified proteins reconstituted in planar lipid bilayers, include protein kinase A, protein kinase C, tyrosine kinase, G- proteins, leukotrienes, cytoskeletal interactions and carboxymethylation reactions. The authors propose that the recently cloned epithelial sodium channel (ENaC) forms the central conducting element of all classes of amiloride-sensitive sodium channels, and that differences in kinetic parameters, ion selectivity, and sensitivity to regulatory factors are due to regional and tissue-specific modulators that associate with ENaC to form heteroisomers differing in stoichiometry and nature of components.

Volume Regulation and Organic Osmolytes in Renal Medullary Cells

As stated so pertinently by Burg in his contribution to the symposium "Homer Smith investigated marine organisms in order to understand better the functions of mammalian kidneys. The wisdom of this approach has been confirmed repeatedly and is evident in any consideration of compatible organic osmolytes in renal medulla". However, organic osmolytes or cell volume regulation are among the few items not mentioned in Smith's monograph The Kidney. To date five organic osmolytes have been identified in renal medullary cells: inositol, sorbitol, glycerophosphorylcholine (GPC), betaine, and taurine. Sorbitol and GPC are synthesized within the cells; for the former the rate of synthesis and for the latter the rate of degradation is controlled by extracellular osmolality. Inositol, betaine, and taurine are taken up by the cells via specific transport mechanisms whose activity is upregulated during hypertonicity of the extracellular medium. The extent of accumulation thereby is postulated to be controlled by the total concentration of all organic osmolytes - irrespective of the concentration of the individual components. The common signal being the intracellular ionic strength which apparently acts as signal for the accumulation [M. Burg, ibid]. According to differential display studies by Gullans et al., renal epithelial cells exposed to hyperosmolar sodium-chloride show an increased transcription of transporters, stress proteins, and metabolic enzymes. In contrast, urea initiates a different and very specific program that appears to involve a urea sensor which activates transcription and translation of the transcription factor Egr-1. Handler and Kwon reported about the identification of a tonicity responsive element (TonE) on the gene for the canine betaine cotransporter that consists of 13 base pairs and is localized upstream to the first exon. It conveys a hypertonicity-inducible increase in transcription to its own or a heterologous promotor in MDCK cells. They also identified a more acute regulation of the transport activity for inositol and betaine. This regulation is probably

posttranslational and involves protein kinase A and C. Activation of both enzymes inhibits uptake of the osmolytes by about 30%.

Organic osmolytes are not only instrumental for the survival and maintenance of the function of medullary cells during urinary concentration but also during the rapid onset of diuresis. When exposed to hypotonicity a rapid release of these osmolytes occurs to counteract the cell swelling. The release occurs probably via channel-like proteins. Goldstein et al. reported on their studies related to taurine release in skate erythrocytes. The release pathway has the properties of a size-limited channel, the osmolyte must be less than 6.3 Å in mean molecular diameter. A positive charge (such as in choline) prevents even smaller molecules to pass. The channel selectivity appears to be based on size selection, taurine (5.5 Å) and betaine (5.8 Å) are transported faster than myo-inositol (6.2 Å). Inhibitor studies using three categories of inhibitors - (a) for anion exchangers (DIDS and PLP), (b) for Cl channels (NPPB, quinine, and MK-447A), and (c) for long chain fatty acids (saturated and unsaturated) further support the idea that the three chemical classes of organic osmolytes are released via the same swelling-activated channel. The anion exchanger band 3 seems to be closely linked to the release process. Studies on DIDS binding and the concentration of dimeric and tetrameric forms in swollen skate erythrocytes suggest that the oligomeric state of the anion exchanger might be directly related to changes in taurine transport. It remains to be determined whether band 3 acts as a channel or transmits the signal of cell swelling to a closely associated osmolyte channel of hitherto undetermined nature. Jackson and Strange have identified by patch clamp measurements swelling-activated anion channels in glioma cells and skate hepatocytes that also transport organic osmolytes, in particular taurine. This anion channel, termed VSOAC (volume-sensitive organic osmolyte/anion channel) is outwardly rectifying, has a unitary conductance of about 50 pS and a novel mechanism of activation. Single channels are switching one at a time from a completely OFF state into an ON state with very high open probability. A similar channel has also been found recently in rat papillary collecting duct cells, as reported by Kinne et al.. The same group demonstrated that at least in IMCD cells the release of each osmolyte is activated by different signal transduction pathways. Sorbitol release is triggered by a transient increase in intracellular calcium which involves arachidonic acid-dependent release from intracellular stores followed by the activation of calcium channels in the plasma membrane. There are further differences with regard to the involvement of G-proteins and the osmotic threshold for activation. In addition, the osmolyte release systems are distributed differently in the apical and basal plasma membrane. Sorbitol and betaine leave the cells exclusively through the basal-lateral membrane, whereas GPC, taurine, and myo-inositol use luminal and contraluminal transporters. The latter two seem to share at least one transport

system. Thus, in contrast to the common signal for the accumulation under hypertonic conditions, the hypotonic release of each organic osmolyte seems to involve very special mechanisms of activation and specific transport systems.

Comparative Physiology of the Kidney*

Homer Smith made many pioneering contributions to the comparative physiology of the kidney and its evolution [H.W. Smith, in: Lecture of the Kidney, Univ. of Kansas, 1943; H.W. Smith, From Fish to Philosopher. Little, Brown, Boston, 1953]. One area of particular interest to him was the function of the aglomerular kidneys that can be found in about 30 species of mostly marine fish. The mechanism by which aglomerular fish form urine is, however, largely unknown to this date. The contribution by Beyenbach and Liu dealt with this mechanism. For the fluid secretion in the proximal tubule they propose that magnesium is actively secreted into the tubule lumen from which it cannot diffuse back into the blood. This transport causes the passive transepithelial secretion of diffusible sodium and chloride via the paracellular pathway. Water follows by osmosis. Since there is flow out of the distal end of the tubule a 'dynamic Donnan system' is maintained, driven by the active transport of magnesium. A mathematical model of tubular electrolyte and fluid secretion confirms this assumption. This model can also be employed to explain fluid secretion in proximal tubules of glomerular sea water fish, and thus describes a common, basic physicochemical mechanism for fluid transport in aglomerular and glomerular fish.

The magnesium to chloride ratio in the elasmobranch urine led Homer W. Smith in a paper published in 1931 [H.W. Smith, Am. J. Physiol. 98, 296-310, 1931] to conclude that the kidneys of elasmobranchs are not the only means to excrete salt. He stated "Since considerably more chloride is absorbed than magnesium (from the intestine) this fact shows that chloride has been excreted by an extrarenal route; and the excretion of chloride from a level of 270 mM per liter in the blood to a level of 500 mM per liter in sea water represents a process of osmotic work ..." This site of excretion has been identified to be the chloride cell of the gills and - where present - the rectal gland, that is analogous to the nasal 'salt' gland of the marine bird and reptiles. The rectal gland of elasmobranchs, in particular of the dogfish Squalus acanthias, has - as summarized by Silva et al. - provided the opportunity to elucidate various steps in the transepithelial transport of chloride and their application to many different epithelia, from amphibian cornea to the mammalian kidney. The uniformity of its cell population, the abundance of transport proteins and the variety of investigative pre-

* Indicates a title of a chapter in The Kidney: Structure and Function in Health and Disease

parations that can be utilized, provide a unique opportunity to examine the nature of chloride transport. The basic mechanism of secondary active chloride transport has been elucidated first in this gland and the molecular entities involved in the different steps have been identified. The regulation of chloride secretion is currently of major interest to the investigators. Silva et al. summarized the stimulatory and inhibitory factors. Volume expansion stimulates secretion in the intact animal. This stimulation is probably mediated by atrial natriuretic peptide (ANP) released from the atria and ventricle of shark heart. ANP, in turn, causes the release of VIP (vasoactive intestinal peptide) from nerve fibers in the parenchyma of the rectal gland. VIP activates the adenylyl cyclase and raises the cAMP level in the gland. This second messenger then enhances chloride secretion by increasing the activity of the luminally located CFTR-like chloride conductance and the basal-lateral Na-K-2Cl cotransporter.

Other related peptides such as the C-type natriuretic peptide (CNP) act directly on the rectal gland and bind to guanylyl cyclase-linked receptors, that have been cloned recently.

Neurotransmitters other than VIP mostly inhibit chloride secretion. This holds for somatostatin which exerts its action on adenylyl cyclase and on a site distal to the generation of cAMP. Bombesin causes the release of somatostatin from the nerves in the gland and is also inhibitory. Neuropeptide Y also reduces chloride secretion - probably distal to the site of cAMP generation. Thus a very intricate and complex pattern of regulation exists where stimulators and inhibitors coexist in nerves within the gland.

Another potent regulator of rectal gland function is adenosine. The cellular and molecular mechanisms of its action were discussed by Forrest. Rectal gland cells have a high density of both inhibitory A1-type receptors and stimulatory A2-type receptors. The former have a much higher affinity to adenosine than the latter. Therefore, adenosine inhibits at concentrations between 10 nM and 1 μM and stimulates chloride secretion at concentrations between 10 and 100 μM. This inhibition is probably an endogenous feedback regulation of chloride transport in the rectal gland. As chloride secretion increases adenosine concentration within the cells rises and adenosine is released by the cell into the interstitial space. There it binds to the high affinity A1 receptors and, in turn, inhibits transport. Thus adenosine functions in the rectal gland as an inhibitory autacoid to link energy demand to energy availability.

The other major salt excreting organ of the marine fish are the gills. The regulation of their transport activity during adaptation to different salinities was discussed by Zadunaisky using killifish (Fundulus heteroclitus) as example. This euryhaline fish can be transferred directly from fresh water to sea water and it survives and adapts to the situation. The signal

received by the chloride cells in the gills to secrete more salt is the sudden increase in plasma osmolality produced by the transition from fresh water to sea water. Changes as small as 12.5 mOsm produce a 25% increase in chloride secretion in isolated preparations. This enables euryhaline fish to move back and forth from brackish water to sea water and regulate precisely their internal milieu.

Another particularly memorable event was the evening lecture at the MDIBL. During a glorious sunset a lobster and clam bake was served, followed by the presentation of Stephen Jay Gould entitled "New Insights from Comparative Biology". After briefly discussing and commenting upon Homer W. Smith's view on evolution he used this opportunity to elegantly and eloquently challenge the commonly held belief that "life history is an at least broadly predictable process of gradually advancing complexity through time" and to point to the important paleontological features that oppose this assumption. They are "the constancy of modal complexity throughout life's history, the concentration of major events in short bursts interspersed with long periods of relative stability; and the role of external impositions, primarily mass extinctions, in disrupting patterns of 'normal' times". According to Gould "these three features combined with the more general themes of chaos and contingency require a new framework of conceptualizing and drawing life's history" and thus he proposed a new iconography of evolution [S.J. Gould, Scientific American 271, 63-69, 1994]. This new iconography postulates that "the maximal diversity in anatomical forms - not in number of species - is reached very early in life's multicellular history". All major stages in organizing animal life's multicellular architecture occurred in a short period, beginning less than 600 million years ago and ending by about 530 million years ago - and the steps within this sequence are also assumed to be discontinous and episodic. "This Cambrian explosion represents an initial filling of the 'ecological barrel' of niches for multicellular organisms. Later times feature extinction of most of these initial experiments and an enormous success within the surviving lines. This success is measured in the proliferation of species but not in the development of new anatomies. Each of these early experiments received little more than the equivalent of a ticket in the largest lottery ever played out on our planet - and each surviving lineage, including our own, inhabits the earth today more by luck of the draw than by any predictable struggle for existence".

Homer W. Smith drew quite a similar conclusion when he stated "There are those who say the human kidney was created" (i.e. did not develop) "... to keep our internal environment in an ideal balanced state. I would deny this - it owes the architecture, not to the design or foresight of any plan, but the geologic revolutions of 6000.000.000 years" [S.J. Farber, ibid].

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Henson, J.H., C. D. Roesener, S. Capuano and R.J. Mendola. Immunolocalization of the kinesin related protein (KRP $_{85/95}$) in the midpiece and flagellum of sea urchin (Strongylocentrotus droebachiensis) and sand dollar (Echinarachnius parma) sperm.....1

Rappaport, R. Width of the contractile region in sand dollar (Echinarachnius parma) egg cleavage furrows.....4

Conrad, G.W., M.J. Janasek, N.M. Martinez, and A.H. Conrad. Mechanisms of silver ion (Ag^{+}) toxicity in fertilized eggs of Ilyanassa obsoleta.....5

Maylie, J., D. Harvey, F.M. Van Dolah, and M. Morad. Maitotoxin activates a non-selective cation channel in cardiac myocytes of Rattus norvegicus and Squalus acanthias.....7

Fan, J., L. Cleemann, and M. Morad. An exocytotic release pathway triggered by Ca^{2+} influx via Ca^{2+} channels in chromaffin cells from Rattus norvegicus.....11

Schafer, J.A., Jr, O. Omulepu, B. Chen, S. Cherukuri, and D.C. Dawson. Micromolar concentrations of inorganic mercury alter membrane conductance of Xenopus oocytes.....15

Conrad, A.H. and G.W. Conrad. Molecular characterization of myosin and the sodium-proton antiporter in Ilyanassa obsoleta.....17

Newton, C.R., J.A. Tilghman, and D.W. Towle. Direct sequencing of PCR-amplified Na^{+}/H^{+} antiporter cDNA from the blue crab Callinectes sapidus.....19

Morrison-Shetlar, A., D. Soto, and B. Wolpin. Partial cloning and sequencing of the renal sodium-D-glucose cotransporter from dogfish (Squalus acanthias).....22

Behnke, R., S. Brill, M. Rao, and B. Forbush. Upregulation of the Na-K-Cl cotransporter protein in saltwater adaptation of the rainbow trout, Salmo gairdneri.....24

Forbush, B. and A. Pernak. Occlusion of ions by the Na-K-Cl co-transporter in the intact rectal gland of the spiny dogfish, Squalus acanthias.....26

Herter, P., M. Elger, B. Kohl, L. Renfro, H. Hentschel, R.K.H. Kinne, and A. Werner. Immunohistochemical localisation of a NaPi 2-cotransport system in the kidney and intestine of winter flounder (Pleuronectes americanus).....28

Baum, M., H. Hentschel, M. Elger, E. Brown, S. Hebert, and H.W. Harris. Homologs of an extracellular calcium/polyvalent cation-sensing receptor (CaR) are localized to the apical surfaces of specific epithelial cells in organs critical for ionic homeostasis in the elasmobranchs, Squalus acanthias and Raja erinacea, as well as teleosts (Pleuronectes americanus), (Oncorhynchus mykiss) and Fundulus heteroclitus.....31

Suzuki, Y.J., J. Monterubio, B.A. Hughes, L. Cleemann, D.A. Abernethy, and M. Morad. Hydrogen peroxide regulates the ryanodine receptor and the Na^{+}-Ca^{2+} exchanger in rat (Rattus norvegicus) cardiac myocytes.....33

Silva, P., R. Solomon, K. Spokes, K. Mooney, D. Gumbs, R. Fraley, and F.H. Epstein. The inhibitory effect of ATP on chloride secretion by the rectal gland of Squalus acanthias is not mediated by the adenosine A1 receptor.....35

Silva, P., R. Solomon, K. Spokes, and F.H. Epstein. The secretion of chloride by the rectal gland of the little skate, Raja erinacea.....36

Solomon, R., P. Silva, R. Fraley, K. Mooney, A. Hoche, D. Gumbs, S. Solomon, J. Boyer, J. Matthews, and F.H. Epstein. Ammonium chloride inhibits chloride secretion in the rectal gland of Squalus acanthias.....37

Hentschel, H. and M. Elger. Renal morphology of the killifish, Fundulus heteroclitus.....40

Zadunaisky, J.A. and L. Au. Effect of hypotonicity on chloride transport in the isolated opercular epithelium of Fundulus heteroclitus.....43

Swenson, E.R., B.C. Taschner, D.S. Miller, T.H. Maren, and J.L. Renfro. Presence of non-hematopoietic carbonic anhydrase in the kidneys of two salt-water teleosts, Pseudopleuronectes americanus and Fundulus heteroclitus.....45

Swenson, E.R., B.C. Taschner, and T.H. Maren. Effect of membrane-bound carbonic anhydrase (Ca) inhibition on bicarbonate excretion in the shark, Squalus acanthias.....47

Claiborne, J.B., J. Campbell, and L. Long. Evidence for an amiloride sensitive gill Na^{+}/H^{+} exchange during the recovery from acidosis in the long-horned sculpin (Myoxocephalus octodecimspinosus).....48

Kormanik, G.A. and C. Harris. Ammonia distribution between blood and tissues in late-term embryos of the dogfish, Squalus acanthias.....50

Preston, R.L., M.T. Kaleta, K.M. Katsma, G. Lapetina, K.A. Simokat, and P.R. Zimmermann. Quinidine inhibits taurine transport by the coelomyocytes of the marine polychaete, Glycera dibranchiata.....52

Preston, R.L., K.M. Katsma, G. Lapetina, and P.R. Zimmermann. Effect of media anion composition on mercury inhibition of taurine transport by the coelomocytes of the marine polychaete, glycera dibranchiata.....55

Miller, D.S., D.F. Croft, and J.A. Zadunaisky. Organic anion uptake by trabecular meshwork (TBM) cells from human eye.....59

Ballatori, N., A.T. Truong, A. Donald, and J.L. Boyer. Volume-regulatory amino acid transport in hepatocytes from Raja erinacea.....61

Fletcher, C.A., A. Donald, N. Ballatori, M. Nathanson, and J.L. Boyer. The development of a method for measuring cell volume regulation in single hepatocytes from Raja erinacea.....62

Davis-Amaral, E.M., A. Acosta, E. Wilson and L. Goldstein. Chloride and taurine effluxes occur by different pathways in Raja erinacea erythrocytes.....64

Musch, M.W. and L. Goldstein. Alteration of ankyrin binding accompanies volume expansion in little skate (Raja erinacea) erythrocytes.....65

Musch, M.W., E.M. Davis-Amaral, and L. Goldstein. Pyridoxal binding sites in volume expanded little skate Raja erinacea erythrocytes.....66

Atherly, L. and D.S. Miller. Vanadate inhibition of organic anion secretion in killifish, Fundulus heteroclitus renal proximal tubules.....67

Nathanson, M.H. and K. Mariwalla. Extracellular ATP and Hg^{2+} mobilize Ca^{2+} from distinct intracellular pools in hepatocytes isolated from the little skate Raja erinacea.....69

Cantiello, H.F., C.F. Jones, A.G. Prat, G.R. Jackson, Jr. ATP content and release in cultured rectal gland cells from the shark Squalus acanthias.....71

Cantiello, H.F., G.R. Jackson, Jr., G. Maguire, and H. Karten. Evidence for an ATP-conductive pathway in retinal neurons of the shark Squalus acanthias.....75

Haynes, J.K., D. Sanderlin, O. Omulepu, R. Lehrich, and J.N. Forrest, Jr. Volume-activated taurine efflux from Squalus acanthias rectal gland cells.....79

Crockett, E.L., S.M. Vekasi, and E.E. Wilkes. Metabolic fuel preferences in gill and liver tissues from freshwater- and seawater-acclimated eel (Anguilla rostrata).....83

Wang, R., L. Wu, Y. Zhang, D. Stea, M.A. Mateescu, A.D. Doyle, and Mc. R. Branch, Jr. The cardiac effects of copper and ceruloplasmin on spiny dogfish shark (Squalus acanthias).....85

Semrad, C.E., D. Seward, and A. Donald. Effect of cadmium on actin cytoskeletal structure in skate (Raja erinacea) hepatocytes.....88

Mulvey, M. and N. Berliner. Role of metalloproteinases in mediating heavy metal toxicity in the dogfish shark, Squalus acanthias.....90

Kidder, G.W., III and P.J. Foster. Acute effects of certain heavy metals on Mytilus edulus.....92

Betka, M. Metallothionein induction during spermatogenesis in Squalus acanthias.....93

Evans, D.H. and M. Gunderson. A prostaglandin, not nitric oxide, is the endothelium-derived relaxing factor in the shark (Squalus acanthias) ventral aorta.....94

Evans, D.H. and M. Gunderson. Cadmium inhibits endothelin-induced, but not acetylcholine-induced, contraction of the shark (Squalus acanthias) ventral aortic vascular smooth muscle.....95

McClusky, L.M., M. Betka, D.S. Miller, and G.V. Callard. Analysis of the apoptotic form of programmed cell death (PCD) during spermatogenesis in spiny dogfish, Squalus acanthias.....96

Van Dolah, F.M., T.A. Leighfield, and L.M. Sugg. Cell cycle regulation in the dinoflagellate, Gambierdiscus toxicus: mitosis is coupled to the diurnal cycle by a blue light dependent signal.....98

Koob-Edmunds, M.M., J.A. Trotter, and T.J. Koob. Identification of stiffening and plasticizing factors in sea cucumber (Cucumaria frondosa) dermis.....101

Keilstein, J.T., H. Stolte, and T.J. Koob. Biomechanical properties of hagfish (Myxine glutinosa) notochord.....105

Koob, T.J. and A. Summers. On the hydrodynamic shape of little skate (Raja erinacea) egg capsules.....108