Molting Hormone Dynamics in the American Lobster, *Homarus americanus* (Milne-Edwards) (Crustacea, Decapoda)

By

MARK JAY SNYDER
B.S. (University of California, Davis) 1981
M.S. (University of California, Davis) 1985

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Endocrinology

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA

DAVIS

Approved:

Committee in Charge

1990

ABSTRACT

Aspects of molting hormone dynamics were followed in the American lobster, *Homarus americanus*. Radioimmunoassay (RIA) measurements of hemolymph ecdysteroid (Ecd) titers showed more variability than that previously reported for other crustaceans. Male lobsters had significantly higher hemolymph Ecd titers in both late premolt and postmolt. The principal metabolites identified by high-performance liquid chromatography (HPLC)-RIA of hemolymph were highly polar Ecds that were excreted primarily in the urine. High polar metabolites consisted of 20-hydroxyecdysonoic acid, 20,26-dihydroxyecdysone, and conjugates of most of the different free Ecds. The active molting hormone, 20-hydroxyecdysone, was the predominate hemolymph metabolite only during the large final hemolymph peak that occurred in late premolt Stages D_2^2 to D_3^1. Urine was the route of most of the measurable Ecd excretion for all molt stages although feces accounted for a larger percentage of the total excretion during mid-premolt (Stage D_1). Fecal Ecds were principally apolar Ecd conjugates, with smaller amounts of high polar and free Ecds. Lobsters were shown to have an efficient "detoxification" mechanism for ingested Ecds which are absorbed by the midgut gland and converted to apolar conjugates prior to excretion in the feces without further absorption to the hemolymph. The apolar Ecds could account for nearly 50% of the total Ecds in the midgut gland in late premolt. The midgut gland proved to be the most important lobster tissue for the absorption and metabolism of Ecds as indicated by injections and *in*

vitro cultures with [^{3}H]-ecdysone. Additionally, a binding protein for these metabolites in midgut glands was found which did not bind any of the free Ecds tested. Its function was hypothesized to be related to directed excretion of the apolar Ecd conjugates in the feces without absorption back into the body. Injection experiments utilizing [^{3}H]-ecdysone demonstrated that lobsters may be able to regulate Ecd metabolism as a means of controlling circulating hormone titers. Evidence was also obtained suggesting that regulation of Ecd excretion rates could be another mechanism by which lobsters could control hormone titers.

ACKNOWLEDGEMENTS

I would like to thank many people for their assistance during my graduate training. First and foremost, thanks are due to my major professor, Dr. Ernest Chang, for all of his knowledge, encouragement, and friendship during my graduate years. I especially thank Ernie for teaching me patience and for much practice in the intricate arts of science. Thanks much Ernie.

I thank my other committee members, Drs. Wally Clark and Bill Lasley, for their interest and care in reading my thesis. Drs. Bill Lasley, Keith Nelson, David Borst, Dorothy Skinner, and Charles Holliday made important suggestions which improved some of my experiments.

I am very grateful to Dr. Walter Bollenbacher for supplying enough anti-ecdysteroid antisera to complete my work. Dr. Malcom Thompson supplied several ecdysteroids which aided in the identification of metabolites in my samples. I gratefully acknowledge Dr. Jin-Hua Cheng for his assitance in working out the problems with our cannulation techniques, and for his friendship during the difficult times. I also thank Art Hertz, Diane Aronstein, and Marilyn Bruce for their help in the laboratory and friendship over the years.

Many of my fellow students were a pleasure to be around, helped support my emotional habits, and kept me sane: Khalida Sabeur helped me survive the rigors of the Endocrine group, Sherry Fitzsimmons has been a great friend and dive buddy, Drs. Bob Okazaki and Nicole Berthelemy-Okazaki helped me through my graduate toilet training,

Henry Fastenau taught me scuba and commaraderie, Claus Brigman and Bob Tamone were fun to dive with, Dr. Chris Ameyaw-Akumfi shared lobster behavior, snooker, and life skills with me, I enjoyed the company of and diving with Bill and Laura Bennett and Rogers-Bennett, Ruth Dotimas provided Philippine insights, and Richard Brantley made my summers more pleasant. Many others at BML made my time here worthwhile, and thanks to those I have forgotten to mention.

Special thanks are due to Dr. Jim Clegg for his support of graduate students at BML. I especially thank him for financial support to attend several ASZ meetings, through the Roger and Elizabeth Sippl foundation. I extend the warmest of thanks to California Sea Grant for their support through a Sea Grant Traineeship. I would have had a difficult time completing my thesis work without their financial support. I thank Eleanor Uhlinger for so much assistance in retrieving articles and doing computer searches. All of her work has saved me countless hours traveling to Davis.

Last of all, I thank several people for their years of love and support. I thank my parents, Joy and Ed, for their constant encouragement over the years. And most of all, I thank my wife Dana DeKoven, for being there when I needed a hug, and sharing both the good and bad moments.....Thank you, Dana.