Understanding Basic Biology of Mammalian Reproduction:

Growth, Development and Mother's Milk

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Summer 1999 Faculty Development Grant Proposal

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I. Scope and Purpose of the Project

For most of my post-baccalaureate years, I have been investigating the basic biology of reproductive systems in mammals. Specifically, I am interested in those animals with variations on the "typical" human system that contribute to our understanding of common, underlying principles that play regulatory roles in mammalian reproduction. For example, I published a series of papers (Kwiecinski et al., 1987a,b) on the extraordinary burden placed on maternal skeletal mineral reserves during pregnancy and lactation in the common North American little brown bat (*Myotis lucifugus*). The unusually large size of the single fetus at birth (30% of maternal post-partum weight) and the relatively short lactational period (18 days at weaning when the young bat attains approximately 80% of adult weight and 95% adult size) are extremely stressful on maternal calcium and skeletal homeostatic systems. In essence, the female sacrifices her skeleton for that of her young's. The most successful and long-lived females are those that satisfy the needs of their offspring by planning ahead and stashing extra skeletal mineral reserves. If only some humans would do the same... This research led to investigations of vitamin D and its role in reproductive skeletal mineral reserves which eventually led to receipt of a NIH-AREA grant during my tenure at the University of Scranton.

Another area of investigation that follows the same general reproductive theme is my recent efforts to characterize growth and development, milk production, and milk composition in a tropical American omnivorous bat, *Phyllostomus discolor*, the lesser spear-nosed bat. I have successfully

established a breeding colony of these bats at The University of Scranton and, for several years, I have collected data for determining growth rates during neonatal development (Nebzydoski and Kwiecinski, 1997). One benefit of such activities has been the ability to supply the Bronx Zoo (Wildlife Conservation Colony) with animals that serve as "seed" bats for their colony of the same species. Despite their efforts, the Bronx Zoo had not been able to establish a breeding colony of this hardy species. I am now positioned for a unique opportunity to further my studies in this species concerning reproductive and life history characteristics, including maternal investment (milk production and composition) during growth and development of young. The value of such information is of interest to biologists in itself, and moreover, the applications and ramifications are far reaching. For example, in many natural populations of plants and animals, there is a critical need to maintain captive breeding populations as necessary refuges for declining world populations. In some instances, not enough has been known about basic life histories to make appropriate management decisions before it was too late for a particular species, as was the situation surrounding the extinction of Pteropodid bats on the Japanese islands (Kunz and Racey, 1998).

For all mammals, milk composition is physiologically controlled by the mother and its nutrient content may be correlated with the nutritional needs of the growing young. Because growth rates of mammalian young are linked with the ability of the mother to provide nutrients, milk composition and output may well represent the best measure of maternal investment during lactation in many mammals. In general, small mammals have large litter sizes and short lactation periods. Rodents, the most numerous small mammals, typically produce small litters that are weaned quickly at 30-44% of adult size (Millar, 1977); however, bats depart from typical small-mammal lactation

strategies. The young are unable to fly and forage until they achieve nearly adult dimensions (Barclay, 1994). Consequently, near the end of peak lactation, mothers must feed young that are nearly adult size (Kunz and Stern, 1995). This high nutrient demand not only affects litter size, but may limit the growth rate of pups as they approach weaning. Since pup growth is dependent on maternal milk production until weaning, milk composition should reflect the nutritional needs of the pup as it develops. Analyzing changes in concentrations of nutrients throughout the lactation cycle yields important insights into the specific requirements of mothers and postnatal growth and development of offspring. To date, data on milk composition are available for only 15 of about 920 species of bats (Kunz et al., 1995; Stern et al., 1997). Furthermore, using a captive colony with offspring of known maternal history, lactation stage, and age avoids the many limitations inherent in collecting samples from wild-caught bats (Kunz et al., 1995).

The purpose of the proposed research for Summer 1999 follows naturally from my current growth and development data and milk sample collections that have been ongoing in my research program. I propose to continue to collect milk samples, and to determine measures of maternal investments, by analyzing milk samples at the Nutrition Research Laboratory, National Zoological Park, Smithsonian Institute, Washington, D.C., under the tutelage of Dr. Olav Oftedal. The study animals are small (ca. 40 g) and the milk samples collected are small (0.05 – 0.5 ml), necessitating microanalyses. This particular lab is the only laboratory in the world equipped to analyze such small milk samples, and I have no alternatives other than to cooperate with Dr. Oftedal at the National Zoological Park. The program that Dr. Oftedal described to me requires that I be present for approximately 4-6 weeks of training with the analytical

instrumentation and another 4-6 weeks to analyze my samples. With a summer faculty grant from The University of Scranton, I could accomplish training and analyze most, if not all, samples. These preliminary data could then be critical components for publications, grant proposals and to direct future research endeavors.

II. Methodology

Milk will be collected from lactating females in the colony at the University of Scranton after 4-6 hours of separation from pups (≥ 72 samples, or one sample each week from 6 - 8 lactating bats over a 12 week period; this is in progress as you read this proposal). Bats are hand-held (and cooperative--no anesthesia required), 100 microliters of oxytocin is given by intraperitoneal injection, and milk is expressed by manual massage into capillary tubes. Collected milk is stored in microcentifuge tubes at -80°C until analyzed. Oxytocin (the natural hormone of milk letdown, bovine source, purchased from Sigma Chem.Corp.) is given to insure a true sample of gland contents. Without oxytocin, milk in deep gland ducts will not be expressed.

Milk samples will be transported to the Nutrition Research Laboratory, National Zoological Park, Smithsonian Institute, Washington D.C., on dry ice. Analysis of milk samples for lipids, carbohydrates, nitrogen, protein, dry matter, and water will be by established standard methods (Oftedal and Iverson, 1995) for samples of adequate size and by testing new technology to miniaturize the assays even further. An inherent problem with the standard methods is the reliance on assays established for larger samples (100 ml – 1 liter) from humans and cows, whereas bat samples are typically much smaller (0.05 – 0.5 ml). Dr. Oftedal's lab recently

purchased a Perkins-Elmer CHN (carbon-hydrogen-nitrogen) Elemental Analysis System that needs validation. Dr. Oftedal has performed preliminary studies that have indicated the CHN system should be valid for the small samples sizes we would like to use (0.01-0.025 ml). We will validate the CHN system for small milk samples by comparison to the standard methods, and if the CHN is satisfactory, we may be able to reduce the amount of milk samples needed for future studies. Theoretically, we should be able to minimize sample size to 0.01-0.025 ml. If we cannot reach this target sample size, we will at least determine the minimum sample size for the new instrumentation and determine the appropriate steps to achieve our target.

III. Product/Result

The data I propose to collect cannot be accomplished at the University of Scranton because of the lack of instrumentation and expertise. Furthermore, the data I have been collecting during the last few years will complement the milk data and provide a detailed analysis of maternal investment during neonatal growth and development. The immediate product will be two data sets describing nutrient concentration in the milk of *Phyllostomus discolor*. One data set will be derived by standard methodology and the other by the CHN system. From these products, at least two very important manuscripts will result. One manuscript will describe nutrient concentrations throughout the lactation period in this species. The other data set will result in a manuscript validating the CHN system for analysis for small milk samples, including comparisons to standard methodology. Also, the data could be applied to outside granting concerns (e.g., NSF) to further involve undergraduate students in the study of the costs/benefits of maternal investment in growth and development of offspring.

IV. Relevance/Utility/Impact

The data proposed to be collected this summer, are relevant in that they provide a large "piece of the puzzle" characterizing growth and development in this species. For years I have been collecting and accumulating physical data on growth and development of neonates. The milk analyses provide a measure of maternal contribution to growth and development, and, with the physical data, I can synthesize a detailed contribution to our understanding of growth, development and maternal investment in these bats, that have unusually large neonates, growth profiles and size at weanling. Also, the data collected will be used for future publication(s) and to prepare a major grant proposal for extramural funding. I have chosen to pursue this project because it offers excellent opportunities towards writing grant proposals with high chances for funding that include undergraduate student participation. In essence, the proposed activities are designed to increase the efficacy of my undergraduate teaching and my professional research programs. My teaching fertilizes my research and vice versa. I believe it is important for students to learn to apply modern analytical techniques to whole animal physiology so they can become aware of the multifaceted approaches to biological investigation.

V. Qualifications of the Applicant

I am a Ph.D. biological scientist with the skills necessary to develop and apply new techniques to the questions of basic reproductive biology as outlined in this proposal (see abbreviated Curriculum Vitae).

VI. Feasibility

To receive training at the Nutrition Research Laboratory, National Zoological Park and to analyze the appropriate number of samples (6 samples for each of twelve weeks of lactation = 72 samples) is feasible within the time frame of one summer. It may take more time to analyze the data, but the hard part of generating data, from instrumentation and expertise not available in Scranton, can only be accomplished during such a time frame. Even with the unpredictable nature of life's circumstances, this project can be brought to fruition during the summer period if enough attention is paid to it. Without the funding requested by this proposal, I will not be able to spend the time in Washington D.C. to do the work. With the support asked for, I expect to accomplish the milk analyses necessary for at least two publications and one grant proposal, which can be written in part while waiting for incubations, equipment performance, etc. while in Washington D.C.

VII. Outside Support

Life support (room and board) while in Washington will be sought through Catholic University and Friends of the National Zoological Park. The proposed project has not received outside support. In order to submit a competitive grant proposal to an outside agency, preliminary data and some indication of success are essential. One of the objectives of this project is to collect such preliminary data and to demonstrate that this research program is feasible.

VIII. Previous Grants

I have not received a University of Scranton Faculty Development Grant in the last three years.

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