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A Few New Developments in Primate Housing and Husbandry

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Introduction

Over the last few decades of the 20th century, laboratory animal scientists have made considerable advances in the development of a vast array of animal models. This has been especially true for the establishment of nonhuman primate models as research resources. For these species, standards of care and criteria for definition, in terms of immunological, behavioral, and genetic factors, have been significantly enhanced in just the last few years. While these advancements are important, they represent only the initial steps toward the levels of care and definition that will be required in the 21st century. This paper will present a brief discussion of the past achievements, current practices, and future aims related to the development of nonhuman primate research resources at one primate facility in the United States. The discussion will focus on the primate colonies at The University of Texas M. D. Anderson Cancer Center (UTMDACC) and our efforts to continue to enhance the definition of our primates and the samples we obtain from them. This philosophy of continuous refinement and definition of nonhuman primate models for research applies to many, if not all, primate facilities in the international laboratory animal community.

A major focus of this paper will be to examine the complementary role that behavioral management strategies play in the establishment and enhancement of well-defined nonhuman primate research resources. I will mention the integral role of behavioral management in the development, maintenance, and enhancement of our rhesus macaque and chimpanzee breeding and research colonies. I will briefly describe why we converted our traditional rhesus monkey breeding colony into a Specific Pathogen Free (SPF) colony (*Schapiro et al., 1994*; *Buchl et al., 1997*). Our current efforts to further define the colony in terms of MHC haplotypes (*Doxiadis et al., 2000*) and to breed for specific haplotypes, thereby making our colony Specific Haplotype Defined (SHD) in addition to SPF (using line breeding and potentially even embryo splitting techniques) will also be described. Additionally, I will discuss the use of positive reinforcement training techniques to obtain conscious and voluntary biological samples (blood samples) from chimpanzees. Conscious blood samples are obtained to improve welfare and to eliminate potential confounds related to unconsciousness, stress, and/or anesthetic agents, thereby enhancing the quality of the samples we use and thus the value of the research and the resource.

Domestic breeding of rhesus macaques in the United States

The rhesus macaque (*Macaca mulatta*) is an important animal model for a variety of investigational areas including, but not limited to: reproduction (*Bercovitch et al., 1998*; *Westergaard et al., 2000*), nutrition (*Verdery et al., 1997*; *Roth et al., 1999*), behavior (*Capitanio, 1999*), infectious disease (*Ruprecht et al., 1998; Sarkar et al., 1999; Baba et al., 2000*), and cognition (*Marcario et al., 1999*). Breeding colonies of rhesus macaques have existed in the United States since the late 1960s (*Neurater and Goodwin, 1972*; *Schmidt, 1972*), and were originally established to provide domestic research resources that would satisfy anticipated demands for monkeys for research purposes, and to some

extent, to relieve pressure on wild populations. Cayo Santiago, an island colony off the coast of Puerto Rico, was established in 1938 and is perhaps the first and most famous of these colonies (*Carpenter, 1940; Rawlins and Kessler, 1986*). While these populations have produced an adequate supply of animals of suitable quality for past investigations (*Rawlins and Kessler, 1986*), current sophisticated investigative needs and personnel safety concerns have identified that a higher quality, better defined macaque model is required (*Doxiadis et al., 2000).*

Herpes B virus, a potentially deadly human health hazard (*Ward and Hilliard, 1994; Weigler et al., 1990*) is endemic in rhesus macaques. This species may also harbor simian retroviruses (*Daniel et al.,* 1984; *Lerche et al., 1994*), including simian immunodeficiency virus (SIV), simian retrovirus (SRV), and simian T-cell lymphotrophic virus (STLV). For the dual purposes of improving human safety when working with rhesus macaques and of improving the quality of the rhesus monkey as a model for immunodeficiency virus research, an effort has been made to derive a population of rhesus macaques that are free of the above mentioned pathogens. This effort, funded by the National Institutes of Health (USA), has led to the establishment of at least six SPF colonies in various parts of the United States, and in general has been quite successful. The UTMDACC SPF colony currently numbers just over 700 monkeys (*Bernacky et al., in preparation*). Further details on the establishment and success of the SPF rhesus macaque breeding program, and the UTMDACC colony in particular, can be found in Schapiro and colleagues (*1994*), Hilliard and Ward (*1999*); Lerche and colleagues (*1994*), Ely and colleagues (*1994*), and Buchl and colleagues (*1997*).

Domestic breeding of chimpanzees in the United States.

The chimpanzee (*Pan troglodytes*) is also an important animal model in a number of areas of investigation including, but not limited to, aerospace (*Keeling, 1974*), behavior (*Hopkins, 1995; Whiten et al., 1999*), infectious disease (*Nehete et al., 1998*), and cognition (*Whiten, 1998*). As was the case with rhesus macaques, this list is greatly abbreviated. Domestic breeding and research colonies of chimpanzees, established for the same reasons as mentioned for the rhesus macaque colonies above, have also existed in the United States for many years (*Riddle et al., 1982; April, 1994*). While no efforts were made to establish SPF colonies of chimpanzees, current research needs for this species are also becoming more sophisticated. Specifically, the attainment of voluntary, conscious biological samples (particularly blood for pharmacokinetic investigations of experimental compounds) is a high priority. Anesthetized samples, previously adequate, are now far less desirable than conscious samples for these pharmacokinetic analyses late in the drug development process. Positive reinforcement training techniques have been used to obtain multiple (7), temporally distinct (at fixed timepoints spanning 24 hours) blood samples from unanesthetized chimpanzees in our colony (*Schapiro et al., in preparation*). Further details on the national chimpanzee breeding and research program and on the UTMDACC chimpanzee colony (150 animals of both sexes and many ages) in particular, can be found in April (*1994*) and Keeling and Alford (*1992*).

Better defined primate research resources

Our current efforts in primate housing and husbandry are aimed at better defining our primate research resources. This means that in order to satisfy the increasingly specific demands of researchers and their projects, we need better animals, better housing conditions, and better samples and sampling techniques. Additionally, critical personnel safety concerns require safer working conditions for those involved in primate care and research. It should be obvious that enhancing the definition of primate research resources will require considerable short term investment, particularly in personnel time. It should be equally obvious, however, that the long term benefits (superior research, since better subjects and samples, and safer working conditions are involved) will rapidly outweigh the initial costs.

Clearly our efforts to provide more explicitly defined nonhuman primate models will necessitate enhanced colony management, including innovative veterinary, genetic, behavioral, reproductive, and immunological management strategies. The establishment of physically and psychologically healthy, genetically diverse SPF colonies of rhesus macaques (*Ely et al., 1994; Schapiro et al., 1994; Buchl et al., 1997*) is a major first step in this direction. Reproductive and behavioral management paradigms aimed at increasing the number of animals expressing particular MHC haplotypes (e.g., *Mamu-A*01, -B*03, -B*04; Knapp et al., 1997; Evans et al., 1999; Dzuris et al., 2000*) and behavioral management paradigms aimed at obtaining voluntary biological samples from primate subjects are clearly additional steps toward increasingly well-characterized animal models for research.

While primate research is difficult to conduct in Scandinavia and many other European countries for a variety of reasons (*Carlsson, personal communication*), it is still possible to work with nonhuman primate subjects in the United States. This is not to say that primate research is easy, inexpensive, or unregulated in the United States. In fact primate research is extremely expensive and quite heavily regulated. There are eight federally funded Regional Primate Research Centers in the United States, as well as a number of other facilities which house and study primates, including our institution (UTMDACC). Most primate researchers and facilities are concerned about the welfare of their primates and subscribe to the philosophy of the 3 R's (Reduction, Refinement, Replacement). Enhancing the quality of their animals as models (*Doxiadis et al., 2000*) allows laboratory animal scientists and researchers to not only Refine the model and Reduce the number of subjects required, but also to increase the value of the research; extremely beneficial outcomes of model enhancement.

The Role of Behavioral Management in Enhancing the Definition of Nonhuman Primate Models

Behavioral management (*Bloomsmith, 1994*) is an important component of the overall colony management scheme in the primate colonies at UTMDACC. For the purposes of this presentation, behavioral management is composed of three interrelated components; environmental enrichment (*Schapiro et al., 1996; 1997*), socialization procedures (*Alford et al., 1995*), and positive reinforcement training (*Bloomsmith et al., 1998*). For additional general discussion of behavioral management strategies at our facility and at other primate facilities see Laule and Desmond (*1998*), Bloomsmith (*1994*), and Desmond (*1994*). The remainder of this paper will focus on how behavioral management procedures can be useful, if not indispensable, in attaining the goals set forth for the production and use of well-defined primate models in laboratory animal science in the 21st Century.

The role that behavioral management strategies can play in attaining more defined animal models will be illustrated with two examples of enhancing the definition of primate research resources that were motivated by research driven needs. The first will be establishing a Specific Haplotype Defined (SHD) population of rhesus macaques and the second will be obtaining voluntary biological samples for pharmacokinetic studies of experimental compounds from conscious chimpanzees.

Establishing breeding colonies of rhesus monkeys of known MHC haplotypes

There is currently great interest in the biomedical community, and particularly among researchers studying immunodeficiency viruses, in establishing populations of rhesus monkey subjects that are not only free of simian retroviruses and Herpes B virus (SPF*; Schapiro et al., 1994; Buchl et al., 1997*), but are also of known MHC types (*Doxiadis et al., 2000*; *Dzuris et al., 2000*). There are a number of reasons for this, with many of the most important related to issues of experimental design. First, monkeys that express particular haplotypes (*Mamu A*01*) are extremely desirable for certain vaccine investigations (*Knapp et al., 1997*; *Dzuris et al., 2000*). Second, experimental and control subjects should be of the same haplotype for these

investigations (Refinement) to eliminate potential confounding variables (*Evans et al., 1999)* and to Reduce the number of subjects required for individual experiments (*Doxiadis et al., 2000*). As discussed above, our rhesus colony is already SPF and genetically well characterized (*Buchl et al., 1997; Ely et al., 1994*). We are in the process of establishing the SHD component of the colony.

Quite obviously, the first step in establishing an SHD colony is to screen all of the animals for the target alleles. While this task is fairly easily accomplished for one or two alleles for which reagents are available, it is considerably more difficult for alleles for which no reagents are available. Since the allele that is currently of the greatest interest to the AIDS research community (*Mamu A*01, Knapp et al., 1997)* can be screened for, we are in the process of identifying those animals in our colony that express *Mamu A*01*. Once the colony has been screened, the actual work of selective breeding and thus, behavioral management can begin.

Based on previous reports (*Knapp et al., 1997*) and preliminary results from our immunogeneticists (*Nehete and Sastry, in preparation*), approximately 20% of our colony is expected to express *Mamu A*01*. Since our goal is to expand the presence of this allele in the colony, we will have to reorganize existing breeding groups in order to establish *Mamu A*01* "positive" groups. Whereas group reorganization sounds like a fairly simple procedure, given the social tendencies of adult rhesus monkeys, it is in fact, a fairly complicated procedure. Reorganizations performed incorrectly or hastily can result in negative consequences for the animals (trauma due to fighting*; Bernstein et al., 1974*) and for the colony (diminished production). In order to minimize these potential negative consequences, an approach to group reorganization employing several phases must be applied. The two primary steps in this procedure include removal of individuals from their current breeding groups and introduction and reorganization of "strangers" into new breeding groups. Assessments of behavior and determination of compatibility of subjects will be required before, during, and after group reorganization. The primary goals of the behavioral management plan thus coincide perfectly with the primary goals of the overall SHD management plan; to establish compatible breeding groups of haplotype defined monkeys that are just as productive as our other SPF breeding groups.

Without providing much detail, the most useful strategy for integrating strange adult rhesus monkeys into new social groups is a gradual one. Using the principles established by Reinhardt and colleagues (*1989; 1995*), the apparatus discussed by Crockett and colleagues (*1997*), and our own previous experience *(Schapiro et al. 1996; 1997*), groups of seven compatible adult females will gradually be established and eventually introduced to a male of breeding age. In addition to haplotype considerations, group composition will be determined based on previously established genetic and behavioral criteria. Groups will be formed in environmentally enriched enclosures to conform to current animal welfare regulations and supplementary enrichment will be provided to help facilitate and potentially hasten compatibility in new groups. Supplementary enrichment will serve as a distraction to prevent aggression and as a stimulant to promote "socially sanctioned" competitive interactions. Positive reinforcement training techniques will also be used during the group formation process to facilitate social integration. Animals will be trained to present for health examinations and veterinary manipulations, to interact affiliatively and/or reproductively with groupmates, and to provide voluntary, conscious biological samples.

Blood samples from conscious chimpanzees

Provision of voluntary, conscious blood samples for pharmacokinetic studies in chimpanzees is a second illustration of the role that behavioral management strategies can play in attaining well-defined primate resources in response to a research-driven motivation. There is currently a demand for pharmacokinetic analyses of proprietary compounds by the pharmaceutical industry. Their subject of choice is the unanesthetized chimpanzee, which provides an extremely humanlike animal model, prior to

release of the compound for use with humans. While it is fairly easy to get multiple blood samples from unanesthetized humans (drug testing companies) and from anesthetized chimpanzees, it is somewhat more difficult to get multiple voluntary blood samples from conscious chimpanzees. Positive reinforcement training techniques can and have been used for just such a purpose.

Pharmacokinetic analyses of compounds frequently entail mutiple samples at a baseline timepoint and again at fairly fixed timepoints after the administration of the compound (15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, etc.). Chimpanzees at our facility have been trained to place an arm in a "blood collection sleeve" (*Laule and Desmond, 1998*) up to seven times in a 24-hour period, providing exactly the type of voluntary, yet temporally precise samples required (*Schapiro et al., in preparation*). Conscious blood samples provide several additional advantages as briefly described below.

Anesthesia in chimpanzees is difficult in the best of circumstances, requiring long periods of induction, long periods for recovery, and social isolation and observation during induction and recovery. Additionally, unless the animals are trained to present for the anesthetic injection, there is considerable negative stress during the "darting" process for the animals and humans alike. Furthermore, anesthetics affect many biological parameters, perhaps even the parameters of interest in certain pharmacokinetic analyses. Voluntary presentation for sampling, on the other hand, once trained reliably, can be accomplished rapidly, involves little if any negative stress, and is unlikely to adversely affect the biological parameters of interest. Although the training process may initially require a substantial investment of personnel time, if target behaviors are appropriately shaped and maintained, and the animals receive multiple repetitions of the target response, then long term benefits are likely to result. These procedures can also be adapted and modified to obtain other biological measurements, including blood pressure, heart rate, salivary variables (sIgA; cortisol, etc.), vaginal swabs, and even ultrasound.

Very briefly, training chimpanzees to give voluntary blood samples requires the following steps. 1) Desensitize the subject to the blood collection sleeve. The blood collection sleeve is a PVC tube mounted on a portable cage front that has a portion cut out that exposes the animal's forearm (cephalic vein), yet prevents the animal from grabbing the technician. Positively reinforce the subject using a clicking sound and desirable food items when he puts his hand in the sleeve. 2) Shape the subject's behavior so that he will grasp the horizontal metal bar at the far end of the blood collection sleeve. Positively reinforce the subject with the clicking sound and food when he holds the bar for progressively longer periods of time. Holding the bar is an absolutely crucial component of the training process, since this is critical both for personnel safety (while holding the bar, the chimpanzee cannot grab the technicians) and for proper positioning to expose the cephalic vein. 3) Desensitize the subject to the supplies and procedures (alcohol swabs, needles, syringes, vacutainers, occlusion of the vein, etc.) used to collect blood. 4) Shape the subject's behavior through successive approximations to tolerate first brief touches with the needle and finally, penetration with the needle and blood collection, while still holding the metal bar. 5) Give the subject a large reward after the blood has been collected and the metal bar released. When subjects have been successfully trained, blood samples can be reliably collected within two minutes and can be repeated up to seven times in a 24-hour period.

Future trends

All of our future goals are related to the continued refinement of our nonhuman primate models for research. For all of our primate populations, we are planning to champion the use of socially housed primates living in ecologically relevant cages/enclosures that functionally simulate natural conditions (social, physical, and occupational) as the most appropriate model.

For the SPF, SHD population of rhesus monkeys, we will continue MHC typing for additional alleles of interest (i.e., *Mamu-A*11, -B*03, -B*04, -B*17; Evans et al., 1999; Dzuris et al., 2000*) as

typing technology advances and the reagents become available. This will eventually lead to selective breeding strategies to enhance a variety of alleles and/or combinations of alleles. The precise alleles selected for emphasis/amplification will be determined based on the research value of the alleles. To further this effort, we are considering the use of assisted reproductive technologies, specifically embryo splitting, to form pairs of identical twins. The obvious goal is to identify specific lines for breeding and enhance the reproductive output of the line by splitting embryos, placing one member of the pair back in the mother and the other in a surrogate female. While this would clearly amplify the number of line bred offspring in our colony, it would also provide a unique opportunity to study the effects of the environment on behavior and physiology, an intriguing proposition for a psychologist. We are in the process of establishing the collaborations necessary to make this strategy possible.

Summary

As the field of laboratory animal science enters the 21st century, continued refinements of the techniques used to manage research animals in captivity are called for. This is especially true for nonhuman primates housed for breeding and research purposes. While previous decades of work have resulted in effective methods for producing, caring for, and using primates in the laboratory, the next decade or two presents a number of new, exciting challenges in the veterinary, behavioral, reproductive, genetic, and immunological management of primates. These challenges primarily relate to our desire to enhance the quality of biomedical research by providing the research community with increasingly well defined animal models. Behavioral management strategies (i.e., group reorganizations to enhance line breeding and positive reinforcement training to obtain conscious blood samples), are essential to successfully address these challenges.

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