International Perspectives: The Future of Nonhuman Primate Resources, Proceedings of the Workshop Held April 17-19, 2002

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International Perspectives

THE FUTURE OF NONHUMAN PRIMATE RESOURCES

PROCEEDINGS OF THE WORKSHOP
HELD APRIL 17–19, 2002

Institute for Laboratory Animal Research

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INTERNATIONAL PERSPECTIVES: THE FUTURE OF NONHUMAN PRIMATE RESOURCES PROGRAM COMMITTEE

Staff

Preface

Nonhuman primates (NHP) continue to play an important role in the research of many human diseases such as malaria and AIDS. As long as NHP are needed for biomedical research, it is essential that suppliers, users and transporters of these animals work together to establish the best standards of characterization and maintenance to ensure that they are treated humanely, used efficiently and that data obtained from experiments on NHP are scientifically useful. Indeed, the harmonization of standards for NHP should allow for effective reproducibility among laboratories throughout the world. In addition, since NHP resources are limited, it is necessary to ensure that adequate conservation practices are considered, and that the quality of the animals used for research is high.

Characterization of the genetics of NHP promises to provide valuable information that may impact the potential use of some species for certain types of studies. For example, with the escalating use of rhesus macaques for AIDS research, it has become important to further characterize the genetic basis of lentiviral infections. In addition, since NHP are used as models for human diseases, knowledge of the genetics will assist researchers in recognizing homology between NHP and human genes as well as give insights into how interindividual variability can contribute to prediction of risk for certain diseases.

The microbiological status of NHP is also critical to research outcomes in these animals as well as to the occupational health and safety of those who work with them. Increased efforts have been initiated to create

specific pathogen-free (SPF) macaque research colonies that have been selectively screened for important simian viruses. In addition to SPF colonies, international standardization of assays utilized for virological assessment of NHP must be addressed.

Finally, there is a crisis with regard to transportation of NHP. Most national and international airline carriers now refuse to transport NHP and, consequently, research and breeding institutions in the United States have had to rely on one of the Chinese carriers for this purpose. In addition to the dearth of transportation sources, there are duplications of national and international regulations for international transport of research animals that must be addressed with the expectations that recommendations for consolidation will be sought.

All of these issues concern scientists, veterinarians and funding authorities from countries that are major users of nonhuman primates for research as well as those from countries that produce and supply these animals. Many of those in the scientific community who direct or support NHP resources or who use these animals for research had expressed a need for addressing these issues on an international level. The Institute for Laboratory Animal Research, within the National Academies, took advantage of its unique position as a focal point for laboratory animal research issues both in the United States and internationally to organize and host a much needed and important workshop. Participants from all over the world gathered in Washington, DC, to discuss critical issues concerning NHP resources. The proceedings from this workshop are reported in the pages of this publication.

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Primate Priorities—An International Perspective

John P. Hearn, MSc, PhD

INTRODUCTION

It is a real pleasure and privilege to open this conference on the future of primate research and the resources required. The challenges and scientific opportunities are enormous and the resources are limited. The study of nonhuman primate systems at all levels, from molecular through systemic, social through environmental, is the closest we can come to experimental investigation of many of the fundamental factors that influence human biology. In the past 50 years, there have been enormous advances in our understanding of primate biology. We now have an array of new research tools and technologies. The rich scientific agenda that we are about to enjoy at this symposium, replete with new data, is proof of these capacities. It is timely to ask how we should set course for the future and what the priorities should be in investing the sparse resources to the best advantage.

Without being overly dramatic, it is fair to say that most of us in this company would not be alive, or would be debilitated, if it were not for the improvements in health that have resulted from primate research. A few simple examples include polio and other vaccines, antibiotics tailored to protect against specific diseases, transplantation and surgical technologies. Some argue that the needs for primate research are now met so that

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our level of knowledge is adequate. In this address, I will argue that the knowledge imperative is never sated, nor the applications exhausted. The more fundamental the question, the wider are the potential applications. Future challenges are just as demanding as those of the past.

One core theme of this conference focuses on the critical situation that is developing globally over the current shortage of rhesus monkeys, exacerbated in recent years by the research needs of the AIDS plague. In aiming to set a framework for the meeting, I will consider briefly a few examples of the continuing research imperatives that require such resources. I will then comment on the requirements for rhesus monkeys, some of the species that might provide alternative model systems, the need for real partnerships between biomedical and conservation research programs, and the ethical essentials in this complex area. Finally, I will suggest a few issues and areas for action. In doing so, I ask how can we make the best use of these few days to influence the future of primate research and resource development, since many of the human primates who will make the decisions are here.

RESEARCH IMPERATIVES

This is a great time in primate biology, when basic and strategic research can often be translated quickly to new applications and solutions that are relevant to health and society. The biotechnologies that are being developed now will fundamentally affect the quality and sustainability of life and biodiversity. Availability of and access to many of these technologies are not universal, however, and differences exist between less developed and developed countries. There are also differences and disagreements due to cultural, religious, and ethical considerations.

A few examples of areas where basic and strategic primate research is now helping to transform our expectations include AIDS and our understanding of infection immunology, stem cells and the knowledge of development and transplantation therapies, and gene therapy based on new functional genetics. The avoidance of age-related debilities based on molecular and cellular health and the enhancing of our understanding of neuroscience, behavior, and learning through imaging provide further exciting options. The opportunities arising from the genomics-proteomics-phenomics sequences are among the most exciting in the history of biomedicine. The monitoring of emerging diseases and their transition between animals (including primates) and humans is of special interest. All of these and many others will depend to an extent on rigorous primate research and validation. The resources to achieve such an ambitious agenda are limited. Therefore we must invest these limited primate resources strategically to deliver the widest possible benefit.

THE RHESUS MONKEY

Due principally to the boost given to rhesus monkey research 50 years ago during the polio pandemic, this species has become the generalized laboratory primate of choice, especially in the United States. Information on the biology of the species is the broadest of any primate other than the human. Due to the lack of availability and relative expense of rhesus monkeys, the cynomolgus macaque has replaced the rhesus in areas such as applied drug development, toxicology, and teratology. These two are the species of choice for much of biomedical and behavioral research as it applies to human health. In some respects this is surprising since aspects of the biology of the rhesus, including its reproductive seasonality, are not similar to the human pattern.

Surveys of rhesus monkeys in their home countries over the past 10 years, including India, reveal increases in numbers. I request respectfully that the Indian government might consider allowing limited access to their country's rhesus resources by their own scientists and by the international community. Even if this access is restricted to the provision of breeding nuclei from which second generation animals can be available for research, it could make a significant difference. Some of the emerging challenges and diseases, including HIV-AIDS, are likely to affect the subcontinent. It may be wise to have the necessary scientific and animal resources to participate in the global response. The rhesus monkeys available from China appear to have variations in their immunology and endocrinology from the Indian animals, making comparisons difficult.

The National Institutes of Health (NIH), and in particular the National Center for Research Resources (NCRR), have given long-term leadership in the development of global primate research. The eight National Primate Research Centers, with 20,000 animals of 20 species, have the largest and most comprehensive program of self-sustaining breeding colonies and specialized research programs in primate biology. This long-term approach is of enormous value and has contributed spectacularly to the advancement of knowledge and the improvement of human and animal health. One factor in this success is the availability of animals of high quality with known pedigrees, and sometimes known immune characteristics, that are available for a very wide range of biomedical and behavioral research, including neurobiology, behavior, genetics, aging, development, reproduction, and many diseases.

The potential for accelerating the breeding of primate stocks, if necessary using assisted reproductive techniques, could be given support within the centers and in collaboration with those in source countries. Greater national and international collaboration among universities, special research centers, and industry could make more efficient use of scarce

materials. An expansion of the World Directory of Primatologists and Primate Infonet could improve the level of communication and provide a greater knowledge of resources and opportunities for collaboration worldwide. This in turn could reduce the overall numbers of primates required in research and assist in the conservation of the species.

The Institute for Laboratory Animal Research (ILAR) of the National Academies has played a major role in developing the norms and standards for animal research, including primates, to the world's best practice. This continuing role is vital into the future if the challenges currently facing us and the constantly changing research needs, including those due to the shortage of rhesus monkeys, are to be overcome.

ALTERNATIVE OPPORTUNITIES

For the historical reasons alluded to above, the rhesus monkey has been established as the major species of choice for biomedical research, with the cynomolgus a substitute for aspects of toxicology. There are other species that hold particular advantage in niche areas of biomedicine and behavior research. Among these, the nearest genetic relatives to the human are the great apes, but their size and sensibility make them unavailable except to a very few researchers, and their use must be restricted to only the most essential applications under stringent controls. The baboons have proved of great value in areas that include surgery, parasitology, and cardiology, but they are large and expensive to keep, as well as being dangerous except under carefully managed conditions. The South American species utilized in biomedicine, including the marmosets, tamarins, owl, and squirrel and cebus monkeys, have particular attributes. The common marmoset is perhaps the most widely used general laboratory primate after the macaques, but this is more evident in Europe than in the United States.

New advances in noninvasive technologies are providing more efficient and rapid ways to develop some biomedical programs. The ability to monitor aspects of the genetics, nutrition, endocrinology, reproductive status, and general health of an animal or human through the analysis of small samples of urine, feces, or blood facilitates the transitions and comparisons between primate and human research. In addition, the great advances in noninvasive sensing, tomography, and imaging technologies now allow repeated studies with minimal stress and damage.

CONSERVATION AND COLLABORATION

An important consideration is that almost all of the primate species studied in biomedical research are not endangered. Many of them, in-

cluding the rhesus, cynomolgus, baboon, and marmoset, are common or even pests in areas of their ranges. Studies on these species can benefit humans in the understanding of systems and the avoidance and treatment of disease. In many ways, these same studies and those on humans can also benefit the nonhuman primates through a greater knowledge of their genetics and reproduction, nutrition and disease, ecology and social organization.

Biomedical and behavioral research accounts for a majority of the rhesus monkeys bred and managed in captivity. Of approximately 20 species, the other species studied are investigated in a wide range of research. In my opinion, it would be of benefit to all of those engaged in these studies and uses, and to the agencies who support them, if 2% of the budget were put toward conservation in captivity and in the wild. Benefits from such an investment would include a greater appreciation of the behavioral and ecological requirements of primates, together with more enlightened methods for maintaining them in captivity. Other benefits should include an increase in field studies, under competitive and well-supervised conditions, which would enable improved conservation, survival, and habitat protection in the wild. These arrangements could be managed jointly with conservation organizations and zoos to obtain best critical mass in meeting biomedical and conservation goals. In a conference such as this, with representatives from all of the major countries engaged in captive and field primate research, there is no better time or opportunity to establish such a principle and to build such networks. Time is not on our side.

ETHICAL ESSENTIALS

The human species faces severe challenges over the next 50 years. The way in which these challenges are approached will determine the survival of many animal and plant species, including primates—and perhaps of the human primate. They include an increase in the human population from the current 6 billion to between 10 and 12 billion by 2050, having already increased from 1.2 billion in 1850 and 2.5 billion in 1950. The forward projections may be affected by AIDS and other emerging diseases, or by famine and disorder, each of which also has an impact on biodiversity.

There is an accelerating pace of habitat destruction, overfarming, habitat islanding, exotic species introductions, and pollution and environmental chemicals that can affect the development, reproduction, fertility, and health of humans and animals. At the same time, our ability for learning and advancing knowledge holds the opportunities for overcoming many of these constraints. We must harness and invest the available

resources to best effect. The global approach argued for above, with research and training collaborations that include the developed and less developed countries, is essential for human and nonhuman primates.

An ethical and suitably regulated framework for primate research is welcomed and supported by all responsible researchers. ILAR has proven world leadership in aspects of these developments and in appreciation of the needs for a continuing dialogue between all concerned, including the public. This small and select conference, open to all stakeholder organizations including representatives of "animal rights" groups, is an example of such foresight. As a strategic reality check of the field, it could be repeated to monitor progress and the achievement of goals every 2 to 4 years, perhaps in conjunction with other primate society meetings including the International Society of Primatologists.

ACTIONS ARE URGENT

In closing, this must not be just another conference that raises issues without looking to actions. The presence of decision makers from around the world makes this an unusual opportunity to find solutions. Let me select and suggest, with respect, the following 10 candidate areas for consideration during the meeting:

- **Global collaboration**—Explore all possible ways to develop research and resource networks that ensure value for investment and economy of materials. Engage researchers especially from nonhuman primate source countries.
- **Basic and strategic research**—Give priority to at least a 30% of budgets investment toward long-term, basic, and strategic research that will fuel the next era of innovation.
- **Emerging issues**—Maintain a strategic involvement as new scientific opportunities and challenges arise. Current examples include emerging diseases and transmission between human and nonhuman primates, microbiology and biodefense, stem cells, and cell therapies.
- **Animal care and welfare**—Adopt policies for continuous improvement in standards, as pioneered and developed by ILAR, but with appropriate adaptations for country conditions.
- **The conservation cycle**—Build synergies and critical mass between captive and field studies in biomedicine and conservation that will benefit both human and nonhuman primates.
- **Funding**—Dedicate 2% of funding toward both captive and field research that advances our knowledge of the primate species most studied in biomedicine and health.
- **Internet development**—Seek innovative ways to make relevant

information in primate biomedicine and conservation available in developed and less developed countries. Expand the activities of Primate Infonet and the World Directory of Primatologists.

- **National primate research centers**—Maintain these centers as a national treasure and as a hub for national and international research and development in primate biology, health, and conservation.
- **Infrastructure**—Where possible, facilitate arrangements for sophisticated analytical equipment, laboratory resources, and information and communications technologies to be available for joint projects between developed and less developed countries.
- **Communication**—Engage in a positive dialogue with all relevant constituencies, explaining the vital need and value of primate research and the advances that are benefiting human and animal health.

ACKNOWLEDGMENTS

I thank Professor John VandeBerg and the organizing committee for inviting me to address this important conference, considering the future of primate research and resources. I thank the NAS, NCRR, and ILAR (especially Joanne Zurlo and Kathy Beil) for supporting my attendance. The opinions expressed in this address are mine and not necessarily theirs.

I also thank the American primatologists, many of them present, with whom I have worked on the issues and toward many of the objectives noted above: David Abbott, Chris Abee, Dick Dukelow, Andy Hendrickx, Ron Hunt, Lorna Johnson, Fred King, Jerry Robinson, Judy Vaitukaitis, John VandeBerg, David Watkins, and Leo Whitehair. I acknowledge my own mentors, Hugh Tyndale-Biscoe and Roger Short. I dedicate this address to Leo Whitehair in recognition of his leadership, humanity, and friendship, much appreciated by all primate biologists.

Session 1

Conservation and Supply, Part 1

Sustainable Utilization of Kenyan Nonhuman Primates for Biomedical and Conservation Research

Jason M. Mwenda, PhD

The Institute of Primate Research (IPR) is a nonprofit institute that was established by the Kenyan government in 1960 to conduct biomedical and conservation research using the East African nonhuman primates. IPR is a designated World Health Organization (WHO) collaborating center for research in reproductive health and tropical diseases and a member of the European Union (EU)-supported Primate Vaccine Evaluation Network. IPR is the only multidisciplinary primate center in Africa.

The Institute of Internal Scientific, Ethics, and Review Committee and Animal Care and Use Committee review all proposals and study protocols before initiation. These committees ensure that during the conduct of the biomedical research, the welfare of nonhuman primates is not compromised and the study protocols conform to the international guidelines on biomedical research using nonhuman primates (NHPs).

IPR is currently involved in research in the following areas: reproductive health, tropical diseases, primate medicine (zoonotic infections, natural and experimental aging processes including Alzheimer's disease), primate behavior, ecology, and conservation. Thus, IPR is involved in research that promotes better health for humans and animals, and especially the NHPs. In this endeavor, the Institute encourages networking with other scientists with mutual research interests and that enhances

Institute of Primate Research, Nairobi, Kenya

local capacity building and promotes regional and international collaboration.

Some notable achievements of the Institute include:

1. Development of the vervet monkey model for cutaneous leishmaniasis. This model has now been accepted by WHO as an appropriate model for testing for vaccines and drugs against leishmaniasis.
2. Identification (for the first time) of malaria-like parasites in monkeys and investigation of monkeys potentially transmitting malaria to humans.
3. Development of the baboon as an ideal model for schistosomiasis research. This model is being used to test the efficacy of candidate vaccines. IPR scientists have shown the highest protection level in schistosomiasis Mansoni using radiation-attenuated vaccine in the baboon model (85% protection level).
4. Development of the rotavirus diarrhea monkey model, which will be valuable for testing rotavirus vaccines to prevent severe diarrhea in children and elucidating the pathogenesis of rotavirus infection/disease.
5. Establishment of diagnostic tests for screening pregnant baboons and women for immunological causes of infertility (caused by presence of antiphospholipid antibodies and endometriosis (characterized by severe pelvic pain).
6. Development toward a new class of birth control methods (antifertility vaccines) for use by both men and women. Available results of testing of these vaccines show anti-CG contraceptive vaccines did not result in adverse effects in female baboons. Similarly, anti-LDH-C4 vaccine for men showed effective fertility control in baboons with no evident side effects. These immunological contraceptives offer advantages over other fertility control methods in terms of efficacy, reversibility, and safety and may be more acceptable in the African cultural setting.
7. Development of vervet monkey and baboon models for testing AIDS drug and vaccines before clinical trials in humans.
8. Recognition of IPR as a center of excellence for biomedical research using primates (monkeys) and specialized training (undergraduate, M.Sc., Ph.D., postdoctoral) and international scientific exchange.
9. Development of effective strategies for conservation of endangered nonhuman primates (monkeys), including De Brazza monkeys, Tana River colobus monkey, and crested mangabey.

Twelve Old World monkey species are found in Kenya. IPR has facilities for maintaining 400 monkeys, which represent nine species.

In Kenya, there is an increasing demand on land for urbanization and agriculture/farming activities, mineral resources, and the establishment

of timber industries. These activities have increasingly reduced the areas available to wild primates. Thus, NHPs are commonly regarded as pests, and farmers often kill the baboons and vervets for crop-raiding. In addition, due to increased community need for forest resources and farming, land pressure on the remaining forest patches has led to the genuine concern for developing effective conservation strategies for the endangered primate species found in Kenya.

IPR, in collaboration with Kenya Wildlife Service and the relevant government departments, has coordinated efforts for conservation of the following endangered and threatened NHPs: De Brazza monkey (*Cercopithecus neglectus*), Angolan black and white colobus monkey (*Colobus angolensis*), eastern black and white colobus (*Colobus guereza*), Tana River red colobus (*Procolobus badius*), and Trana River crested mangabey (*Cercocebus galeritus*).

In Kenya, the threatened De Brazza monkeys are confined to the Kakamega Forests and the Trans Nzioa plain, which lies east of Mt. Elgon and west of the Cherangani Hills. The endangered Tana River red colobus and crested mangabey are both endemic to forest patches along the lower Tana River.

In recognition of the need to conserve these two primate species and their unique habitat, the Kenyan Government gazetted 171 km^2 in 1976 and established the Tana River Primate Reserve (TRPNR). Ongoing conservation studies by IPR scientists in TRPNR are focused on (1) carrying out primate census and determination of distribution of primates, (2) evaluating changes in forest sizes in relation to populations of red colobus and crested mangabey, (3) assessing human and natural impacts in forests along the lower Tana River, and (4) developing management and conservation strategies for the endangered red colobus and their habitat along the lower Tana River.

Overall, the Kenyan government and IPR encourage primate conservation programs that encompass community participation, primate translocation, and conservation of biodiversity that is geared toward sustainable utilization of natural resources.

Supply and Use of Nonhuman Primates in Biomedical Research: A South African Perspective

Jürgen Seier, PhD, MSc

GENERAL

There are six indigenous nonhuman primate (NHP) species in South Africa, of which two are used in biomedical research: the chacma baboon (*Papio ursinus*) and the vervet—or African green monkey (*Chlorocebus aethiops*). This presentation concentrates on these two species.

GEOGRAPHICAL DISTRIBUTION

Baboons occur in most parts of South Africa and across the entire country, with the exception of the driest areas, and they can be found near major centers such as Cape Town. Vervet monkeys are much less widespread than baboons and are mainly confined to the eastern parts of South Africa, with pockets in a few other locations. Vervet monkeys also occur in and near major centers such as Durban. Both species occur in all neighboring countries including Namibia, Botswana, Lesotho Zimbabwe, Swaziland, and Mozambique.

CONSERVATION

The general conservation status of chacma baboons and vervet monkeys is "low risk" (Rowe 1996), and neither species is considered threat-

Primate Unit, Medical Research Council, Tygerberg, South Africa

ened in South Africa, with the exception of certain local populations (e.g., Cape Peninsula baboons). Although both species are considered "common" in many areas where they occur, a lack of national census does not allow any firm conclusions. A proposal for a national census is currently being produced. Vervet monkeys and baboons are protected in conservation areas, but the protection and status outside these areas, particularly in view of agricultural problems, vary in different provinces.

The controlling bodies for wild and captive populations, importing and exporting (CITES), and even moving across provincial borders are the nature conservation departments. To provide some perspective, the populations in 1981 were estimated to be approximately 20,000 to 25,000 vervet monkeys and 3,600 baboons in all protected areas of Kwa Zulu Natal (Bourquin 1981), one of nine provinces in South Africa. However, apart from being dated, such figures are misleading since many NHPs live outside protected areas, and in some provinces, baboons occur in considerably larger numbers.

CONFLICTS AND THREATS

As in many other countries with wild populations, in South Africa, NHPs raid agricultural crops and may vandalize gardens and homes, where human development has encroached on their territory. Farmers destroy primates that become agricultural pests, and where the territory of NHPs is close to urban areas, a number fall victim to car accidents every year.

As an example, the baboons of the Cape Peninsula, which are an isolated population of about 350, are a major tourist attraction. The peninsula baboons frequently forage on the shore and supplement their diet with seafood. Residential and other human development has encroached on their territory to an extent that in certain areas, baboons have taken to raiding gardens and homes regularly. Presently the mortality rate outstrips the birth rate. In some scenic spots, visitors have been regularly feeding baboons, resulting in the baboons being habituated to people, who exacerbate the problem of large, wild primates near residential/urban areas.

USE OF NONHUMAN PRIMATES IN BIOMEDICAL RESEARCH IN SOUTH AFRICA OVER THE LAST 3 YEARS

Although baboons are used mainly, both baboons and vervets are utilized in all research fields requiring primate models. Of the few primates used in biomedical research, approximately 210 baboons and 120 vervet monkeys have been utilized annually in 17 facilities nationwide. In

the last 21 years, there has been a 81% decline in the use of baboons and a 88% decline in the use of vervet monkeys.

SUPPLY OF NONHUMAN PRIMATES

Over the last 3 years, about 3% of baboons and 60% of vervets were captive bred, and the rest were caught from the wild. Presently only one facility systematically breeds African NHPs (vervet monkeys) for research. Local nature conservation authorities must issue permits for trapping NHPs, and proof of agricultural damage by such primates must be provided in some provinces. Only authorized trappers receive permits.

Research facilities maintaining NHPs are inspected annually by nature conservation authorities and must apply every year for holding permits, even in the case of captive-bred primates. In some provinces, nature conservation authorities require an ethically approved study protocol and proof of agricultural damage before permits for capture are issued. Over the last few years, there has been an increased reluctance by the nature conservation authorities to issue capture permits. However, due to the small number of primates used locally, there are usually no major supply problems from local sources.

Although there may have been sporadic transfers of small numbers, there is no provision to other countries and South Africa is not an exporter of NHPs. Moreover, it would be very difficult and costly, if not impossible, to move NHPs from South Africa since all airlines that operate transcontinentally from South Africa do not transport primates destined for biomedical research.

PRIMATE FACILITIES IN SOUTH AFRICA

Local facilities, which are designed for small numbers of wild-caught primates and not for breeding or long-term maintenance, concentrate on rodents. However, if funding became available, the potential of establishing breeding centers does exist, and there is generally an excellent research infrastructure and expertise.

There are no national or regional centers, and the largest South Africa facility maintains 250 to 300 vervet monkeys, with the capacity to produce 100/annum. This facility has the infrastructure for long-term maintenance and has been breeding vervet monkeys for about 25 years, which has progressed to the second and third generation.

Other facilities typically maintain between 20 and 60 NHPs, mainly baboons. There are no primate centers in neighboring countries and little, if any, use of primates in biomedical research.

CONCLUSIONS

- Vervet monkeys and chacma baboons occur in many parts of South Africa and across the entire Southern African region.
- Like NHPs in other source countries, vervets and baboons face a variety of threats and are in conflict with some human activities in South Africa, but these factors are unlikely to endanger the entire population.
- Some of these threats are highly localized (e.g., the Cape Peninsula baboons).
- Although mostly wild-caught primates are used in biomedical research in South Africa, the numbers are small and the effect on wild populations is minimal if any.
- Most research centers have no facilities for long-term maintenance and breeding of nonhuman primates, although the potential to establish such centers exists.
- South Africa has not been exporting NHPs with the possible exception of some sporadic transfers of small numbers.

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Sustainable Primate Resources Through SPF Breeding Programs in Indonesia

Joko Pamungkas, DVM, MS, and Dondin Sajuthi

In response to conservation concerns, both nationally and abroad, over the status of Indonesia's naturally occurring primate populations, the Indonesia Department of Forestry enacted a regulation in 1994 that restricts the export of nonhuman primates to progeny from captive/managed breeding facilities. The Forestry Department also applied a law governing the quota of wild-caught primates that can be used as breeder replacements or for research to be conducted in country.

Along with these government policies, the Primate Research Center at Bogor Agricultural University, in Bogor, Indonesia, in collaborations with several national and international institutions (e.g., Washington National Primate Research Center) has established two breeding facilities in Indonesia. An island natural habitat breeding facility supporting an introduced population of simian retrovirus (SRV)-free *Macaca fascicularis* was initiated in 1987 on Tinjil Island located off the south coast of West Java, and an SRV-free *M. nemestrina* captive-breeding facility in Bogor was initiated in 1992. Progeny from these two breeding facilities have been utilized in biomedical research programs in Indonesia and worldwide.

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Use of Nonhuman Primates in Biomedical Research in India: Current Status and Future Prospects

A. J. Rao, PhD

India is known for its floral and faunal diversity. This diversity is best represented by the nonhuman primate (NHP) population found in the country. Of the more than 30 genera and 130 species of NHPs known throughout the world, as many as eight genera having 10 species and 38 subspecies are known from India alone (Parthasarathy 1995). Of these species, seven are exclusively Indian in their distribution: *Ananthana ellioti*, *Tupaia nicobarica*, *Macaca assamensis*, *Macaca radiata*, *Macaca silenus*, *Semnopithecus johni*, and *Semnopithecus geei*. An additional five species are restricted to the tropical, dry, and moist deciduous evergreen forests of peninsular India: a shrew-like prosimian, a slender loris, two macaques, and a langur. Indian monkeys belong to one family, Cercopithecidae, with two subfamilies, Cercopithecinae (the macaques) and Colobinae (the langurs).

None of the human-like great apes are found in India. The only tribe of apes inhabiting India is the gibbons, of which a single species, the hoolock, is found in the forests of Assam and Chittagong. The total population estimate of *Hylobates hoolock* is approximately 170,000 (Mackinnon and Mackinnon 1987). *Macaca silenus* (lion-tail macaque) is found in the evergreen forests of Western Ghats in India. This species is endangered

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and its numbers are declining fast. The estimate is less than 2500 as of 2000 (World Conservation Union 2000). It is suggested that this species seems to be unable to adapt to human settlement.

Another endangered species in the Nilgiris (the Blue Mountains) is the langur, *Semnopithecus johni*, which is also found in the dense evergreen forests of Western Ghats in India. The golden langur, *Semnopithecus geei*, and the capped langur/leaf monkey, *Semnopithecus pileatus*, are found in the dense forests of Assam. It is reported that there are approximately 950 km^2 of suitable habitation (decreased from about 1500 km^2 in the early 1970s) available for the golden langur in India, with an estimated population of less than 2000 (Choudhury 2001).

The two species of loris monkeys found in India are the slow loris, *Nycticebus coucang*, and the slender loris, *Loris tardigradis*. The slow loris is found in the forests of Assam, and the slender loris is restricted to South India (Prater 1980). Despite the fact that several genera and species of NHPs are found in India, detailed studies on the numbers in the wild and some aspects of biology are available for only three macaques: *Macaca mulatta* (rhesus monkey), *Macaca radiata* (bonnet monkey), and *Semnopithecus entellus* (the common langur or hanuman monkey). Of these three, the bonnet monkey is restricted to the southern states of Karnataka, Andhra Pradesh, Kerala and Tamil Nadu, and the rhesus is found in North India and also in some parts of Andhra Pradesh, a southern state. However, the common langur is distributed all over India from the Himalayas to Cape Comarin. Of these three macaques, the most widely used species for biomedical research throughout the world and in India is the rhesus. Estimates of the numbers in the wild range from 300,000 to 500,000 (Malik 1999; Prater 1980), and they are distributed all over North India and some parts of Andhra Pradesh in South India.

Demographic studies suggest that rhesus monkeys in India are increasing (Imam and Yahya 2001; Sally Walker, Zoo's Outreach Organization, personal communication, Chennai, India, 2002). The increase is obvious from the fact that in several cities in North India, they have become a menace, threatening children and the elderly and snatching food. They are known to damage crops and property (Imam and Yahya 2001), for which they are trapped, hunted, and even subjected to government-sponsored extermination campaigns. However, the main reason for these animals to invade urban, populated areas is the loss of their natural habitat owing to population explosion and subsequent spread of agriculture and teak, coffee, and tea plantations. As a consequence, there is severe competition for food, and these animals have invaded human settlements where they sense availability of food.

Orthodox Hindus consider monkeys as sacred animals to be revered and protected, and quite often they feed these animals near temples. How-

ever, if this trend continues, their numbers will increase in the populated areas, and the subsequent decrease in their natural habitat will eventually result in a situation involving steps to reduce their numbers. All of this calls for a comprehensive management plan for these commensals. Another undesirable fallout of this urbanization problem is that these monkeys, due to close human contact, have become susceptible to diseases, parasitic infection, and so forth and are therefore unsuitable for research studies unless they have been certified to be free of diseases (Malik 1999). In view of this situation, there is an urgent need to restore their natural habitat, at least in certain densely populated areas.

The other macaque that is widely distributed in South India is the bonnet monkey, *Macaca radiata*. Although the rhesus monkey was the animal of choice for research, thanks to the efforts of Prof. Moudgal and his colleagues during the last 40 years, the bonnet monkey is now being used in several laboratories in India. Over the years, considerable information on the biology, hormonal profiles, and breeding husbandry of bonnets has become available (Rao and others 1998.). An official estimate of the bonnets in the wild was provided by Kurup and colleagues (RCSRUP 1981). In this study, the estimate of bonnets, rhesus, and langurs in South India was found to be 310,000 (bonnets: 170,000; rhesus: 50,000; langurs: 90,000). Although this estimate is almost 20 years old, regional demographic studies indicate that the number of bonnets is also on the increase. Estimates in 2002 are more than 100,000 for rhesus and approximately 200,000 for bonnets (Sally Walker, personal communication, 2002). A study of population parameters revealed that the bonnets are well adapted to commensal life around human settlements. They are better tolerated because they are less aggressive than rhesus.

As regards the hanuman langur, demographic parameters indicate a low reproductive profile (RCSRUP 1981). The population of langurs appears to be declining. The current estimates range around 29,000, which is roughly one third the 1981 estimate (Sally Walker, personal communication, 2002). One possible reason is that these monkeys are not highly adaptable and are generally shy and withdrawn, living away from human settlements. With loss of natural habitat, they must compete for the limited habitat and food, unlike rhesus and bonnets, which have adapted to life in human settlements.

Of the three species of monkeys that have been used for biomedical research in India, a large breeding colony of rhesus was maintained at the Central Drug Research Institute, Lucknow, in 1980, where animals were used for studies on reproductive biology and toxicology. Currently, however, no breeding is undertaken there. The other places where rhesus are maintained are at the All India Institute of Medical Sciences, New Delhi; National Institute of Immunology, New Delhi; Post Graduate Institute of

Medical Education, Chandigarh; National Institute of Virology, Pune; and National Institute of Nutrition, Hyderabad. The animals are used mostly for reproductive biology studies, contraceptive testing, vaccine development, and immunology.

The total number of rhesus monkeys currently under experimentation in various laboratories in India does not exceed 300. Procurement is only from the wild, and no captive breeding is undertaken in any of the laboratories mentioned above. The only place langurs are maintained is in the Department of Zoology, University of Rajasthan; and even there, the animals are used for establishing the normal reproductive parameters such as testosterone levels, sperm counts, menstrual cyclicity, hormonal profile, and some reproductive biology studies such as contraceptive testing. It is reported that it has not been possible to breed langurs in captivity, and the total number of animals maintained in laboratories does not exceed 50.

The largest colony of bonnets is at the Primate Research Laboratory at the Indian Institute of Science, Bangalore. When financial support was maximal, more than 500 animals (both wild caught and colony born) were maintained there. However, the total number of animals maintained at present is only around 200, of which 50% have been colony born. Over the last 20 years, an average of 20 births per year has been recorded (Table 1). As in the case of other centers, the animals are mostly used for reproductive biology studies. The other laboratories where bonnets are maintained are the Institute for Research in Reproduction, Bombay, and the National Institute of Immunology, New Delhi. At both of these places, animals are merely maintained, and no captive breeding is undertaken. The total number of bonnets at these two places does not exceed 150.

Thus, the total number of monkeys used in various laboratories is approximately 700 (rhesus: ≈ 300; bonnets: ≈ 350; langurs: ≈ 50). In addition, a small number of animals (not exceeding 5-10) are maintained for behavioral studies. Relative to the availability of NHPs for biomedical research, the main problems are the restrictions imposed by the Committee for the Purpose of Control and Supervision of Experiments on Animals, the government body that supervises animal research. Demographic data and projected mortality rates for NHPs (particularly for rhesus and bonnets in the wild) indicate they can be trapped and used for research without endangering their survival.

It should be noted that demographic studies indicate replenishment of breeding population at a rate of 31% for bonnets and 36% for the rhesus monkeys in Southern India. In the case of rhesus, it is also reported that the net annual population turnover could be tentatively placed at 17%. On extrapolating this figure to bonnet monkeys, it is felt that a rate of one third the breeding population replenishment can be considered a safe

TABLE 1 Fertility Status of Female Bonnet Monkeys in the Controlled Breeding Program, Primate Research Laboratory, Indian Institute of Science, Bangalore

Year	No. Mated	No. That Became Pregnant After Exposure to Male (%)			Total Pregnancy (%)	No. of Deliveries
		1st	2nd	3rd		
1981-1982	17	10 (58.8)	4 (23.5)	—	82.3	14
1982-1983	45	27 (60.0)	7 (15.5)	3 (6.6)	82.1	37
1983-1984	28	18 (64.3)	4 (14.3)	1 (3.5)	82.1	23
1984-1985	35	20 (57.1)	8 (22.8)	1 (2.8)	82.7	29
1985-1986	27	16 (59.2)	5 (18.5)	—	81.4	21
1986-1987	52	31 (59.6)	9 (17.3)	2 (3.8)	80.7	42
1987-1988	47	27 (57.4)	8 (17.0)	3 (6.3)	88.8	38
1988-1989	38	23 (60.5)	4 (10.5)	3 (7.8)	78.9	30
1989-1990	30	18 (60.0)	6 (20.0)	—	80.0	24
1990-1991	28	17 (60.7)	6 (21.4)	—	82.1	23
1991-1992	45	27 (60.0)	5 (11.1)	4 (8.8)	79.9	36
1992-1993	17	11 (64.7)	3 (17.6)	—	82.3	14
1993-1994	28	18 (64.2)	4 (14.2)	1 (3.5)	81.9	23
1994-1995	35	20 (57.1)	8 (22.8)	1 (2.8)	82.7	29
1995-1996	07	03 (42.8)	2 (28.5)	—	71.4	05
1996-1997	12	05 (41.6)	2 (16.1)	2 (16.1)	74.9	09
1997-1998	05	02 (40.0)	1 (20.0)	—	60.0	03
1998-1999	08	04 (50.0)	2 (25.0)	—	75.0	06
1999-2000	20	12 (60.0)	3 (15.0)	2 (10.0)	85.0	17
2000-2001	17	08 (47.0)	3 (17.6)	2 (11.7)	76.4	13
2001-2002	06	03 (50.0)	1 (16.6)	—	66.6	04
						440

NOTE: 440 births in 21 years = 20.95/year.

harvesting level, allowing for likely adult mortality. This replenishment would be 10% for bonnets and 12% for rhesus populations. In view of these rates, an annual quota of 5000 for bonnets and 2000 for rhesus in South India, and definitely a much higher number for rhesus in North India, can be considered safe for trapping from the wild. However, government restrictions do not permit captures from the wild, and legal regulations stipulate that NHPs procured from government-recognized breeding centers can be used only for biomedical research. Paradoxically, there are no recognized NHP breeding centers, which essentially means that no NHPs are legally available for research. In addition, even if such a center were started right away, it would be at least 5 to 10 years before an animal

would become available for research. Thus, although sufficient numbers are present in the wild, they are not made legally available for use and no animals are available from captive breeding. Finally, even if these restrictions are lifted, there is an urgent need for an organized procurement center for trapping NHPs in the wild, which ensures minimum mortality and safe transport without disturbing the ecological numbers in the wild.

Currently, transportation of monkeys takes place in a rather unscientific manner. There are no cages designed for transport with minimum inconvenience, in terms of freedom of movement. The animals are usually transported in lightweight cages made of iron or bamboo sticks in trucks during the night to avoid heat. Our own experience has been that of transporting monkeys in cages with metal rods, one animal per cage in trucks that reach their destination by overnight journey. During transport, animals are provided with pelleted feed and fruits in addition to cucumber, which serves as a source of water. However, lack of data on the pedigree, age, and disease burden of these wild-caught animals is a problem that must still be faced and solved. One point to be considered seriously is that even if the animals become available from the wild, it is desirable to have national primate centers as in the United States, where captive breeding can be undertaken. Such breeding would ensure that animals are of known pedigrees instead of the often unreliable wild-caught animals.

Considering the scenario described above, no NHP animals are currently exported to any country from India, although before 1977, a total of 500,000 NHPs (including rhesus [80%], bonnets [15%], and hanuman langurs [5%]) were exported from India (Report of Zoological Survey of India 2002). The controlling authority for export of NHPs is the Ministry of Commerce, which makes decisions in consultation with the CPCSEA and wildlife authorities. Under the circumstances, the only way for making the NHPs available for research is to permit their initial capture from the wild, both for research and for starting breeding centers at selected national primate centers. In time, the numbers caught from the wild can be reduced as sufficient numbers from the captive breeding become available. This strategy is essential due to the fact that with an increase in the number of NHPs in human settlements, they are also more prone to infections, thus enhancing the threat of transmission of potential diseases because of increased contact with humans. They are therefore not very suitable for biomedical research. It is pertinent to note that in one study screening of more than 2000 rhesus monkeys captured in Himalayan foothills, more than 40% tested positive for at least one potentially harmful disease (Malik 1999). These data are very alarming given the fact that the animals originally were forest dwelling, with limited contact with humans.

In view of the situation described above, the possibility of monkeys in close contact with human settlements testing positive for several human diseases is quite high. One way to overcome this problem is to relocate these monkeys in batches, where forest cover is available, or in sanctuaries, where deforestation is prohibited. However, it has been suggested that these monkeys should be relocated only in complete or social groups (Malik 1999). Another way to overcome this problem is to increase their numbers by captive breeding. Earlier experience has revealed that both bonnets and rhesus can be very successfully bred in captivity, and attempts should be made to obtain more information on the husbandry of the hanuman monkey.

Available census data clearly indicate that both rhesus and bonnets procured from urban settlements can be used after appropriate quarantining and hence can be made available for biomedical research. If this approach is not implemented soon, there will be disastrous consequences. In this connection, it is pertinent to quote one of the famous conservation biologists in India, Iqbal Malik (1999):

> In India, three primate species in particular are strongly commensals, namely the bonnet macaque (*Macaca radiata*), the hanuman langur (*Semnopithecus entellus*) and, of course, the rhesus (*Macaca mulatta*). Despite the damage they are capable of causing, monkeys have benefited from India's tradition of veneration for them. Nevertheless, in India, as elsewhere, the damage caused by them can put human endurance to the severest test. This will be particularly true in the years to come given that the country's human population will double in a mere 35 years. Perhaps the species that will be most affected by increasing public disaffection will be the rhesus, since most of them live in sites located near or even within human settlements. Besides, the traditional veneration of monkeys could be lost forever. The rhesus could soon be regarded as intolerable and labeled vermin to be destroyed. It is also possible that public disaffection for rhesus could spread to some of the other primate species. In this worst-case scenario, public support for all conservation projects involving monkeys, commensal and non-commensal alike, could suffer (p 27-29).

In conclusion, effective management is a task that demands flexible planning by the national agencies/government bodies and much public support.

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Initiative for Primate Resources, Biomedical Research, and Conservation in Nepal

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The Natural History Society of Nepal (NAHSON) is a scientific and professional organization registered under the rules of the Nepali government and working for the conservation and management of biodiversity of Nepal. NAHSON has a number of different subgroups working in various fields of flora and fauna in Nepal. It also consists of a primate study group headed by the author and working for the conservation and management of wild populations of nonhuman primates in Nepal.

Beginning in 1997, NAHSON and the Washington National Primate Research Center at the University of Washington (WaNPRC) established a collaborative relationship through frequent discussions with and subsequent visits by Dr. Randall C. Kyes, Head of the Division of International Programs of the WaNPRC. This initial relationship developed into a formal collaborative program with the signing of a Memorandum of Understanding (MOU) by both parties on July 2001. This Collaborative International Program in Primatology is expected to result in the establishment of the Nepal Primate Research Center (NPRC).

PRIMATOLOGICAL RESEARCH IN NEPAL

Among the more than 200 known nonhuman primate species worldwide, three species of monkeys are reported from Nepal. Rhesus mon-

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keys (*Macaca mulatta*) are found freely ranging in the wild as well as in urban religious places. Langur monkeys (*Semnopithecus entellus*, formally *Presbytis entellus*) are found freely ranging in the forest and wild marginal areas. The ecological and social behavioral research on the langur monkeys of highland Nepal was conducted during the mid-1970s (Bishop 1979), and the langur monkeys of the subtropical Sal (*Shorea robusta*) forest were studied around 1990 (Chalise 1995). These two species are common and widely distributed from tropical (Terai) to subalpine (high mountains to 4000 m) regions of Nepal (Bishop 1979; Chalise 1995; Southwick and others 1982). The third primate species, the Assamese monkey (*Macaca assamensis*), is reported to range from the midhills to the high-mountain forests of Nepal (Chalise 1999, 2000; Jackson 1990). The data on major behavioral patterns and habitat description of Assamese monkeys of the Makalu-Barun area are available (Chalise 1997, 1998); however, ecological and behavioral details of this species are limited. Ecological and behavioral research of rhesus monkeys in Nepal began during the 1970s in Kathmandu Valley at religious spots (Southwick and others 1982). More recently, preliminary data on the population status of these three primate species of Nepal have been published (Chalise and Ghimire 1998).

MONKEYS AND MOUNTAIN PEOPLE IN NEPAL

The rhesus monkey, found widely in wild and urban areas, is considered a "common" species in Nepal. As a result of decreasing habitat and increasing human population, the rhesus has become a nuisance in the urban areas and is considered an agricultural pest in the mountain regions of Nepal (Chalise 2000, 2001a; Ghimire 2000-2001). Due to the heavy crop raiding habit of this species in the midhills and around the highland protected areas, local farmers have tried repeatedly to wipe out the rhesus populations in these locations (e.g., Mankha and Bhadaure VDC, LNP). For example, Upreti (1985) reported that wild animals in Langtang and Rara National Park raid buckwheat and barley. Jackson (1990) also recorded damage to crops by monkeys in the southern boundary of the Makalu-Barun area.

Crop loss from monkeys is a common occurrence and has a very acute impact on the food supply of the hill people. Several times, local people have attempted to launch mass killing programs to get rid of those "pest" species. The villagers of Mankha and Sindhupalchowk are thought to have killed approximately 500 monkeys in their forest areas in April 1998. They formed a monkey killing squad armed with local knives, clubs, and shotguns to chase monkeys from their surrounding forest. Their complaint was extensive crop losses due to an excessive monkey population.

Similarly, the people of Bhorle VDC (Bhadaure, Rasuwa in LNP 2000) organized a joint effort to chase the monkeys from their area in September 2000. They sought support from other VDCs such as Dhaibung, Laharepauwa, Tupche, Gerkhu, and Bidur Municipality in chasing the monkeys up to a protected forest of Kathmandu (Chalise 2001b).

In light of the increasing conflict between the humans and monkeys, especially rhesus, it is essential that efforts be made to ease this problem. The people of Nepal should be supported to protect their crop field while the wild monkeys should be managed in a sustainable way with the appropriate conservation of their habitat. The conflict between the human interests and primate activities can be managed through the conservation and sustainable use of primate resources of mountain areas. Such an outcome can be achieved in Nepal, as declared by the International Year of the Mountains 2002, through the collaborative primate program between NAHSON and the WaNPRC. The program, outlined below, will not only help to reduce the problems faced by the mountain people but will also allow us to utilize this "common" species, in a conservationally sound manner, to improve the welfare of human beings through biomedical research.

COLLABORATIVE INTERNATIONAL PROGRAM IN PRIMATOLOGY

As noted above, NAHSON and the WaNPRC completed a formal MOU in July 2001 to establish a long-term collaborative international program in primatology in Nepal that will lead to the development of the NPRC. The goals of the collaborative program are:

1. To effect the sustainable development of Nepalese primate resources;
2. To develop the capability of utilizing primates for increasing biomedical research pertinent to human health problems of Nepal;
3. To establish a core group of experts in primatology in Nepal; and
4. To ensure the conservation of naturally occurring primate populations and other biodiversity in Nepal.

The program goals are further outlined in the objectives described below.

Primate resources. To establish breeding facilities in Nepal to provide rhesus macaque progeny (*M. mulatta*) for use in biomedical research at the NPRC-NAHSON, the WaNPRC, and other collaborating Nepali

institutions (e.g., Nepal's Department of National Parks and Wildlife Conservation, [DNPWC] and Tribhuvan University [TU]).

Research. To facilitate an active program of collaborative research with faculty, students, and staff from the NAHSON, the WaNPRC, and other collaborating Nepalese institutions (e.g., TU and DNPWC).

Training. To provide educational and training opportunities in primatology for faculty, students, and staff from the NAHSON, the WaNPRC, and other collaborating Nepalese institutions (e.g., TU and DNPWC).

Conservation. To assist with efforts to manage and conserve naturally occurring primate populations and other biodiversity throughout Nepal.

The program will operate within specific parameters for administration, funding support, primate resources, research, training, and conservation. The principles of the operation are described below.

ADMINISTRATION

The NAHSON will serve as the central organization under which all program-related activities in Nepal will be based. The NAHSON is a nongovernmental organization established in the Kingdom of Nepal to promote the conservation of Nepal's biodiversity. Matters such as administrative office space will be selected/provided by NAHSON in consultation with WaNPRC. The WaNPRC is a National Primate Research Center supported through the National Institutes of Health (NIH) and located at and administered through the University of Washington. All program-related activities in Nepal (including administration and finance, breeding, research, education and training, and conservation) will be under the direction of Dr. Mukesh Kumar Chalise, Head of the Primatology Program in Nepal-NAHSON.

PRIMATE RESOURCES

A breeding colony of rhesus macaques (*M. mulatta*) will be established in Nepal at a site selected by the NAHSON in consultation with the WaNPRC and the DNPWC of His Majesty's Government of Nepal. Support facilities including quarantine, veterinary facilities, and microbiology laboratory (virology, bacteriology, and parasitology) also will be established to support colony screening and clinical care. Progeny born in the colony will be available for biomedical research in Nepal and will be sent to the WaNPRC for advanced biomedical research as needed. The transport (both domestic and foreign) of animal tissue and/or live ani-

mals as part of this collaborative program is subject to the prevailing rules and regulations of the respective governments. Treatment of the animals will be consistent with the principles expressed in the most recent *Guide for the Care and Use of Laboratory Animals* (NRC 1996).

TRAINING

Educational and training opportunities in primatology will be made available to faculty, students, and staff from the NAHSON, the WaNPRC, and other collaborating Nepalese institutions as funds allow. Special emphasis will be given to funding support for Nepali students and researchers to participate in continuing education and scientist exchanges at the University of Washington. Field training programs and short courses also will be developed in Nepal to facilitate education and training in, for example, primate behavior and biology, population management and conservation, primate veterinary medicine, genomics, virology, and microbiology. Both the NAHSON and the WaNPRC will select all trainees and training activities with agreement.

FUNDING SUPPORT

Funding for the collaborative program may come from both private and government agency funds originating from either country. Funding secured by the WaNPRC, either private or governmental (e.g., from the NIH), will be administered and disbursed directly by the WaNPRC for use in the development and support of the Nepal breeding facility and associated research, training, and conservation activities as described below.

RESEARCH

The NAHSON, the WaNPRC, and other collaborating Nepali institutions will engage in collaborative research in areas such as infectious disease (e.g., AIDS and hepatitis C), genomics, behavioral biology and ecology, and conservation biology as funds allow. The collaborative research process should facilitate the development of the scientific infrastructure in Nepal (e.g., via research experience and equipment donation) and transfer of knowledge.

CONSERVATION

The NAHSON and the WaNPRC will conduct and support such activities as primate populations surveys, conservation and management

programs, outreach programs for local citizens, and conservation activities benefiting other biodiversity throughout Nepal as funds allow.

NEPALI GOVERNMENT APPROVAL

To proceed with this collaborative International Program in Primatology (and the establishment of the NPRC), NAHSON and WaNPRC submitted a formal program proposal to the Nepali government in December 2001 for approval to conduct the program-related activities outlined above. The Ministry of Forests and Soil Conservation has reviewed the proposal, and formal government approval is expected shortly.

PROGRAM ACCOMPLISHMENTS TO DATE

- July 29, 2001—NAHSON and WaNPRC sign an MOU to establish a collaborative International Program in Primatology.
- August 2001-January 2002—Dr. Mukesh Chalise travels to the WaNPRC as a Visiting Scientist and Fulbright Scholar.
- December 3, 2001—Formal program proposal submitted to the Nepal Ministry of Forests and Soil Conservation for government approval.
- January-March 2002—Dr. Randall Kyes travels to the NAHSON (and Tribhuvan University) as a Visiting Scientist and Fulbright Scholar.
- February 2002—Drs. Kyes and Chalise, in collaboration with staff from the DNPWC, conduct the first annual field training program (and wildlife survey) in Conservation Biology at Langtang National Park, Nepal. More than a dozen park rangers from around Nepal participate in the training program.
- April 2002—NAHSON and the Nepal Field Study Program (at the University of Washington, UW) sign an MOU to establish educational field training opportunities for UW students in Nepal and exchange opportunities for Nepali students at UW.

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Chinese Primate Status and Primate Captive Breeding for Biomedical Research in China

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Nineteen species of primates are distributed in China, including three families and six genera (Table 1). There are perhaps 21 species of Taiwan macaque (*Macaca cyclopis*, only distributed in Taiwan with 7000 individuals) and douc langur (*Pygathrix nemaeus*, only recorded in Hainan in 1892) are counted. Loris (*Nycticebus* spp.) and gibbons (*Hylobates* spp.) mainly range in Yunnan, with some in Guangxi and Hainan. Langurs (*Presbytis* spp. and *Trachypithecus* spp.) are mainly in Southwest China and Guangxi. Snub-nosed monkeys (*Pygathrix* spp.) are in Southwest China, with some in Gansu, Shaanxi, and Hubei. The species of macaques have wide distribution in China and range mostly south of Yellow River. The stumptail macaque (*Macaca arctoides*) is in Yunnan, Guangxi, Guizhou, Jiangxi, Hunan, Guangdong, and Fujian; the Assamese macaque (*Macaca assamensis*) in Yunnan, Guangxi, and Tibet; the pigtail macaque (*Macaca nemestrina*) in Yunnan; the Tibetan macaque (*Macaca thibetana*) is endemic to China and in Southwest and Middle China, southern parts of Gansu, and Shaanxi, Guangxi, Fujian, and Zhejiang; and the rhesus macaque (*Macaca mulatta*) has the widest distribution in China and is mostly in the areas to the south of Yellow River but mainly in the southern part of China.

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All species of primates in China are protected by the Wildlife Protection Law of China and are listed in the China Red Data Book. Only three species of macaques (*M. arctoides*, *M. thibetana*, and *M. mulatta*) are the second class protected wildlife of national importance, and the rest are all first class, which are strictly forbidden for hunting and killing. Four species of macaques are vulnerable, and the rest are endangered according to the China Red Data Book. These species are also in the 2000 IUCN Red List of Threatened Species (Table 1). Clearly, it is difficult to be optimistic about the status of Chinese primates. There are more than 10,000 individuals in these five species, and only limited numbers of stumptail macaque and rhesus macaque are allowed to be caught for commercial purposes in China. The main threats to primates are loss of habitat, habitat fragmentation, human-caused mortality, lack of knowledge about population numbers and status on which to base sound management decisions, and lack of management to limit mortality to sustainable levels and to conserve necessary habitat.

Almost all primate species in China inhabit forests or areas with forests (especially natural forests). China's long exploitation history is mostly in forest areas, which were once suitable for agriculture but are now gone forever. The forests have decreased to 14%, with much damaged or young secondary growth. At least 70% of the primate habitats have been lost in the last 50 years with population growth, logging, reclamation, settlements, and so forth. Habitats have become fragmented and smaller, and some geographical populations have disappeared. Some primate species were once used for local traditional medicines (e.g., *Presbytis francoisi*), pelt animals (e.g., *Pygathrix bieti*), pests (e.g., *M. thibetana*), or as pets (*M. mulatta*). Such human-caused mortality has resulted in their endangered status in China.

China has paid great attention to wildlife protection in past 20 years, with the development of international biodiversity conservation. The key to wildlife conservation is to protect its habitat. To complement the Wildlife Protection Law, there are 1276 nature reserves covering a of total 123 million ha and occupying 12.44% of the total territory of China. One fourth of these reserves are related to primate conservation. Some of the 1050 forest parks occupy 9.8 million ha, and the primates' habitats, which also play an important role in their protection. All of these natural areas protect a majority of endangered and rare wild fauna and flora species and their habitats, protecting 20 million ha natural forests, which occupy 14.6% of the total areas of forest. Since 2000, the Chinese government has banned any logging on natural forests in the National 10th Five-year Social and Economic Development Plant. The State Forestry Administration has implemented the National Natural Forest Protection Project and has begun the National Wildlife Conservation and Natural Reserves Construc-

TABLE 1 Wild Primates in China

Common Name	Scientific Name	Distribution
Slow loris	*Nycticebus coucang*	West, Southwest, and South Yunnan, Southwest Guangxi
Intermediate slow loris	*Nycticebus intermedium*	South Yunnan
Lesser slow loris	*Nycticebus pygmaeus*	South Yunnan
Stumptail macaque	*Macaca arctoides*	Yunnan, South Fujian, Hunan, Jiangxi, Guizhou, Guangxi, and Guangdong
Assamese macaque	*Macaca assamensis*	Southeast and South Tibet, South and Northwest Yunnan, South Guangxi
Rhesus macaque	*Macaca mulatta*	Most areas south of Yellow River
Pigtail macaque	*Macaca nemestrina*	Southwest and South Yunnan
Tibetan macaque	*Macaca thibetana*	Sichuan, Chongqing, and surrounding areas of Middle and South China
Hanuman langur	*Presbytis entellus*	South Tibet
Francois's leaf monkey	*Presbytis francoisi*	Southwest and West Guangxi, Northeast and Southwest Guizhou, Southeast Yunnan
Phayre's leaf monkey	*Trachypithecus phayrei*	South Yunnan
Capped leaf monkey	*Trachypithecus pileatus*	Northwest Yunnan
Black snub-nosed monkey	*Pygathrix bieti*	Northwest Yunnan, Southwest Tibet
Gray snub-nosed monkey	*Pygathrix brelichi*	Fanjing Mount. of Guizhou
Chinese snub-nosed monkey	*Pygathrix roxellana*	South Sichuan, Gansu and Shaanxi; West Hubei
Black gibbon	*Hylobates concolor*	South Yunnan, Southwest Guangxi, Hainan Island
Hoolock gibbon	*Hylobates hoolock*	West Yunnan
White-handed gibbon	*Hylobates lar*	South Yunnan
White-cheeked gibbon	*Hylobates leucogenys*	South Yunnan

[a]Ma, S. and Y. Wang. 1988.
[b]Wang, S., ed. 1998.
[c]E, endangered; V, vulnerable.
[d]EN, endangered; VU, vulnerable; LR/cd, lower risk/conservation dependent; LR/nt, lower risk/near threatened; DD, data deficient.

Estimated Number		Red Data Book[c]		Protected Class	
1980s[a]	1998s[b]	China[c]	IUCN[d]	China	CITES
1500-2000		E		I	II
	500	E		I	II
300-500	rare	E	VU	I	II
70,000-90,000	70,000	V	VU	II	II
8000-10,000	8000	V	VU	I	II
260,000	200,000	V	LR/nt	II	II
900-1,000	900	E	VU	I	II
100,000 ?	10,000	V	LR/cd	II	II
?	1000	E	LR/nt	I	I
6400-7600	7000-8300	E	VU	I	II
11,000-17,000	11,500-17,000	E	DD	I	II
500-600	500-600	E	VU	I	I
800-1000	2000	E	EN	I	I
300-500	750	E	EN	I	I
10,000	25,000	E	VU	I	I
470-500	500	E	EN	I	I
250-400	200	E	DD	I	I
30-40	30	E	LR/nt	I	I
80-100	40	E	DD	I	I

tion Project, which includes 15 Rescuing Wildlife Programs (including primates and more than 100 endangered species), planning to establish 500 more new nature reserves and wetland conservation areas by 2010. Implementation of these projects should dramatically increase the ability to conserve wildlife resources and their habitats, and especially to maintain and even expand the populations of the most endangered wildlife. However, half of the primate species in China are critically endangered and have narrow ranges, and it will be difficult to restore and protect them.

There are five species of primates used in China for biomedical research. Two species, the crab-eating macaque (*Macaca fascicularis*) and the common marmoset (*Callithrix jacchus*) are alien. The other three species, pigtail, stumptail, and rhesus macaques, are indigenous and had wild populations of 900, 70,000 and 200,000 individuals respectively, in the 1990s (Table 1). The common marmoset (≤95 individuals) is bred in captivity only at Tianjin Medical University and is used for biomedical research. The stumptail and pigtail macaques are not common as laboratorial animals and are currently used only for pharmaceutical tests on hair growth in China. These two species (total of 115 individuals) are raised only in Kunming Zoological Institutes and the Shared Animal Health & Technology (Beijing) Co. Ltd., where they are bred in captivity. The main threats to them are forest destruction and illegal hunting.

The main primate species used for biomedical research in China are the crab-eating and rhesus macaques. The former was introduced into China in the late 1980s, mainly from Vietnam (Cambodia, Laos, and Myanmar) through border trading, and has been bred in captivity very successfully. China has now established several self-sustaining populations with more than 47,000 individuals. The rhesus macaque, the traditional species for biomedical research worldwide, also has been bred in captivity successfully since the 1950s. There are several self-sustained populations in China, with more than 20,000 individuals (Table 2).

The rhesus macaque is abundant in China in the wild and is estimated to total approximately 200,000 presently. It is reported that there are about 10,000 in Guangdong (including Hainan Island), 30,000 to 50,000 in Guangxi and Guizhou separately, 50,000 to 60,000 in Yunnan, and 30,000 to 40,000 in other provinces. Compared with the status in 1950s, its population has greatly declined, as much as 70 to 80%. The main threats are habitat loss and being hunted as pests (Liu 1998).

According to the authors' investigation in 2002 (Table 2), the 23 primate captive breeding farms (PCBFs) for biomedical research in China are in (number in parentheses) Beijing (2), Shanghai (3), Yunnan (3), Guangxi (8), Guangdong (4), Jiangxi (1), Zhejiang (1), and Jiangsu (1), with 46,932 individuals of crab-eating and 19,888 rhesus macaques. The halves of

colonies are breeding groups. In 2001, 15,657 baby crab-eating macaques and 5312 baby rhesus macaques were born in captivity in 23 PCBFs, with birth ratios as high as 33.36 and 26.71%, respectively.

The rhesus macaque has been exported from China since 1984, and the crab-eating macaque since 1990. The main import countries are Japan, the United Kingdom, France, and the United States; and others include Sweden, Switzerland, Netherlands, Spain, South Africa, and Canada. China exported 6765 crab-eating macaques and 2363 rhesus macaques in 2001. Compared with 2000 and 1999, the export numbers increased 4.9 and 35.8% in crab-eating macaques, and 22.1 and 115.2% in rhesus macaques. In China, the export increase has brought about a great advance in PCBFs, which have successfully collaborated in management, exportation, and captive breeding. Eight PCBFs of Guangxi are managed as one group by the Guangxi Laboratorial Primate Research Center, with 4161 rhesus macaque individuals and 29,687 crab-eating macaques in captivity. The Shared Animal Health and Technology (Beijing) Co. Ltd., together with Kaiping Yuejing Rare Animal Farm in Guangdong and Ningbo Primate Farm in Zhejiang, owns 3494 rhesus macaques and 3978 crab-eating macaques. The Yunnan National Laboratorial Primate Center owns 3040 rhesus macaques and 7200 crab-eating macaques. Some of them not only breed primates for biomedical research and exportation but also establish laboratories for foreigners to come to China for primate biomedical research. These data indicate that China has great potential in the exportation of these two species. The data show export of 44.5 and 43.2% of rhesus macaques and crab-eating macaques individuals, with breeding populations as large as 10,871 and 23,704 individuals of the two species respectively, which have reproduced 5312 and 15,657 rhesus and crab-eating macaque babies in 2001, respectively.

Although crab-eating and rhesus macaques are available from PCBFs in China, it is difficult to apply for the permits for catching wild primates for biomedical research according to the Wildlife Protection Law. Some species, especially the first class protected species of primates, are never allowed to be caught from the wild. Permits to catch the second class protected species of primates from the wild must be issued by wildlife management authorities in the province, who often refuse because they are endangered in the provinces. In addition, because the price of monkeys from PCBFs is quite high for many Chinese researchers, some research institutes breed primates themselves to support their biomedical research.

TABLE 2 The Captive Breeding and the Exportation of Rhesus Macaque (*Macaca mulatta*) and Crab-eating Macaque (*Macaca fascicularis*) for Biomedical Research in China in 2001[a]

Province and Captive Breeding Farm	Total[b]
Total in China	19888/46932
Beijing	4463/2754
Shared Animal Health & Technology (Beijing) Co. Ltd.	2619/2418
Laboratorial Animal Center of Chinese Academy of Military Medical Sciences	1844/520
Shanghai	690/48
Shanghai Physiological Institute of Chinese Academy of Science	520/0
Shanghai Jinshan District Agricultural Sideline Company	170/0
Shanghai National Research Center for Safety Evaluation on New Medicines	0/48
Yunnan Province	4809/7346
Yunnan National Laboratorial Primate Center	3040/7200
Kunming Zoological Institute of Chinese Academy of Science	751/123
Primate Center of Biomedical Institute of Chinese Academy of Medical Sciences	1018/23
Guangxi Province	4161/29687
Guangxi Laboratorial Primate Research Center	492/2978
Pingnan Xiongsen Laboratorial Primate Breeding and Development Co. Ltd.	2860/8129
Yulin Hongfeng Laboratorial Animal Breeding Farm	0/3018
Fangchengang Primate Center	386/4538
Guangxi Yinglin Monkey Farm	0/5363
Beihai Monkey Farm	0/3692
Hezhou Wildlife Rescuing Center	423/ 1560
Longzhou Monkey Farm	0/409
Guangdong Province	2774/7097
Gunagzhou Tianhu Endangered Animal Institute	843/1531
South China Endangered Animal Institute	692/2056
Guangdong Shunde Labaratorial Animal Institute	1180/1950

Breeding Group		Individuals Weighing	Annual	Export Number (Year)		
Male	Female	2-5 kg	Birth[b]	2001	2000	1999
2139/3794	8732/ 19910	7529/14948	5312/15657	2363/6765	1935/6451	1098/4980
273/165	1619/1003	1614/1050	1131/849	354/6	516/0	290/20
138/135	969/913	780/725	721/774	226/0	240/0	146/20
135/30	650/90	834/325	410/75	128/6	276/0	144/0
195/24	195/24	300/0	70/0	10/0	14/0	0/0
145/0	145/0	230/0	56/0	10/0	10/0	0/0
50/0	50/0	70/0	14/0	0/0	4/0	0/0
0/24	0/24	0/0	0/0	0/0	0/0	0/0
356/628	1828/2856	2390/3195	1121/2130	600/1872	760/2100	500/1750
211/607	1270/2809	1235/3140	750/2100	600/1872	760/2100	500/1750
68/12	257/43	287/35	168/26	0/0	0/0	0/0
77/9	301/4	868/20	203/4	0/0	0/0	0/0
313/1837	1876/12106	1157/8380	1208/10260	946/3530	248/3554	0/2352
43/129	250/863	67/1254	170/759	157/460	100/596	0/442
200/550	1213/3364	926/2107	764/2926	694/1100	148/1246	0/950
0/272	0/1620	0/423	0/1425	0/450	0/292	0/842
25/309	140/1613	137/1509	91/1322	65/200	0/192	0/0
0/330	0/2668	0/919	0/2301	0/900	0/850	0/0
0/130	0/1373	0/1268	0/1043	0/240	0/378	0/118
45/ 57	273/ 345	27/887	183/ 289	30/ 130	0/0	0/0
0/60	0/260	0/13	0/195	0/50	0/0	0/0
557/1140	1861/3921	1026/2323	930/2418	393/1357	397/797	308/858
120/200	723/1331	500/800	396/783	251/496	137/236	62/168
30/81	308/780	218/596	224/650	136/692	160/440	150/450
400/750	780/1200	250/400	280/500	6/169	100/121	96/240

continues

TABLE 2 Continued

Province and Captive Breeding Farm	Total[b]
Guangdong Kaiping Yuejing Rare Animal Farm	59/1560
Jiangxi Province	710/0
Rhesus Farm of Guanshan National Nature Reserves	710/0
Jiangsu Province	1465/0
Xishan Zhongke Laboratorial Animal Co. Ltd. of Suzhou	1465/0
Zhejiang Province	816/0
Ningbo Primate Breeding Farm	816/0

[a]Deadline for data collection was December 22, 2001.
[b]The babies lower than 1000 *g* in weight and younger than 6 months old were not counted.

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Breeding Group		Individuals Weighing 2-5 kg	Annual Birth[b]	Export Number (Year)		
Male	Female			2001	2000	1999
7/109	50/610	58/725	30/485	0/0	0/0	0/0
310/0	400/0	216/0	102/0	0/0	0/0	0/0
310/0	400/0	216/0	102/0	0/0	0/0	0/0
100/0	700/0	480/0	550/0	60/0	0/0	0/0
100/0	700/0	480/0	550/0	60/0	0/0	0/0
35/0	253/0	346/0	200/0	0/0	0/0	0/0
35/0	253/0	346/0	200/0	0/0	0/0	0/0

The Breeding of Naturally Occurring B Virus-free Cynomolgus Monkeys (*Macaca fascicularis*) on the Island of Mauritius

Mary Ann Stanley, BSc

Mauritius, a tiny island nation in the Indian Ocean, has long been known to international conservation bodies for its extinct and threatened native fauna and flora. In addition to the human, one of the great culprits of this destruction has been the cynomolgus monkey. Portuguese and Dutch sailors introduced cynos to Mauritius from Java around the early 17th century. They proliferated quickly because they had no natural predators apart from humans, and the wild population is now estimated to be between 40,000 and 60,000. Native forests in Mauritius have been greatly affected by the activities of the cynos. They predate seeds of native plants while propagating seeds of exotic weeds that, by growing much faster, slowly choke out the native forest. They are officially recognized by conservation bodies to have contributed to the near extinction of the Mauritian green parrot and pink pigeon due to direct nest predation. Sugarcane growers and fruit and vegetable planters have always treated the Mauritian cyno as a pest because hundreds of hectares of cultivation are lost yearly to this very destructive animal. Sugarcane plantation owners employed people specifically to patrol the perimeter of their estates that border wooded areas, to try and keep monkeys out.

In 1985, Bioculture Mauritius Ltd. (BCM) was established with the aim of using this feral cynomolgus resource to produce naturally occurring B virus-free feral as well as quality captive bred monkeys for export for biomedical research purposes. Virus-free Mauritian monkeys are believed

Bioculture (Mauritius) Ltd.

to have originated from the ones introduced by the sailors as pets. They would have been collected as babies for ease of handling and taming and hence would have been free of sexually transmitted viruses. In addition, the long and difficult boat trip from Java at that time would have acted as a further screen because sick animals would have died en route.

Having established the value of Mauritian cynos, BCM set up breeding groups using feral animals that were removed from areas where they were causing major damage. With trapping areas a maximum of 1 hour's drive from the farm, the animals caught in manually operated traps reached the site the same day with minimal stress. However, to manage trapping areas better, BCM subsequently set up Joint Ventures with Land Holders. BCM is an ISO certified company that, together with its Joint Ventures, has a workforce of more than 200 people including the following five categories: eight veterinary staff, 16 animal technicians, 25 animal handlers, 70 animal caretakers, and six technical staff. The regulatory bodies in Mauritius that control this industry are the

- Ministry of Agriculture;
- Government Veterinary Services;
- National Parks and Conservation Unit (CITES); and
- Ministry of Industry.

Breeding females are held in groups containing 40 to 45 females with two to three males. Environmental enrichment is accorded a very high priority, and BCM employs a full-time ethnologist to act as its Animal Welfare Officer. Through the officer's observations, standard operating procedures are established so as to minimize stress and maximize welfare at all levels of production.

Weaned captive-bred animals are kept at a separate site where they are housed in the same peer group as in the breeding cages. This arrangement has contributed significantly to minimizing postweaning stress. All sites are capped at a maximum number of heads for ease of management, and new sites are constructed.

Feral monkeys coming from the wild are of course housed on separate quarantine sites again where they stay for a minimum of 3 months

TABLE 1 Breeding and Export Statistics

Total number of breeders	~5000
Captive bred production year 2001	3572
Number of feral animals exported yearly (all to the US)	2000

before being transferred to BCM's quarantine site for export or for further testing before being placed in breeding colonies.

BCM's company policy is to grow at a rate of between 5 and 10% yearly, with a percentage of this growth in F2 production. Mauritius, with its introduced population of cynos, is a special case regarding removal of wild-caught monkeys for breeding. Both conservationist and government authorities want this status to continue. BCM's commitment to increase F2 production is linked solely to UK Home Office requirements.

As we face an increasing transport problem in this industry, BCM has amassed much experience together with our distributor Charles River in organizing charters. To date, we have sent six dedicated charters of monkeys from Mauritius to Houston.

Primates for 21st Century Biomedicine: The St. Kitts Vervet (*Chlorocebus aethiops*, SK)

Frank Ervin, MD, and Roberta Palmour

The emergent need for primate studies in all areas of biomedical development exceeds the current demand. The frequency with which therapeutic innovation progresses from mouse to human without primate testing sends chills to those of us old enough to remember the thalidomide catastrophe. A new generation of vaccines directed at "self" epitopes for the treatment of cancer, atherosclerosis, Alzheimer's disease, and so forth—but not tested in primates—provides a recipe for trouble. This exciting area of biotechnology is only one of many where adequate primate studies will soon be mandated by common sense, if not by government regulation. Let me list a few:

1. Drug effects on children;
2. Drug and biological effects on fetus;
3. Phase Zero efficacy testing in primates before proceeding to human trials, ideally in appropriate disease models; and
4. Rapid assessment of safety and immunogenicity of new vaccines.

That there is not the expected demand for such studies reflects several factors: Primate studies are perceived as being very expensive, and this is

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true for many sites. The very high costs quoted are the product of several factors, in addition to greed. These factors include:

1. The wasteful use of many primates for terminal toxicological studies with no attempt to minimize numbers via rational pharmacogenomics;
2. The use of Asian macaques with enzootic herpes virus B, which demands safety garb for personnel and insurance premiums that artificially inflate costs;
3. The failure to select species appropriate to the question at hand or, within species, to select individuals with optimum sensitivity to the experimental intervention; and
4. The failure to follow the dicta of the 1960s Primate Centers Program and the subsequent recommendations for the development of adequate laboratories in countries of origin so that supplies could match needs more precisely.

Looking at contemporary biomedicine, both academic and industrial, there would seem to be needs for the following kinds of primate subjects:

1. Those with spontaneous models of a human disorder (e.g., *M. arctoides*' patchy alopecia, to provide the hair growth industry with a fortuitous model). Colon cancer occurs in the cottontop marmoset and human, and rarely in other primates. Hypertension occurs in *C. aethiops* and lagothrix and rarely in other nonhuman primates. Atherosclerosis, rare in *C. aethiops*, is common in *Papio*.
2. Those with inducible disease models (e.g., squirrels are very sensitive to vitamin C deprivation; *M. nemestrina* is more responsive to laboratory SIV infection than other primates tested; and hepatitis B sensitivity apparently remains restricted to the human and the chimpanzee).
3. Those with some particular phenotypic advantage. Baboons are large enough for a variety of surgical interventions. They also have an immune response profile close to that of the human with respect to porcine xenotransplantation. They are therefore much preferred over the Asian macaques. *C. aethiops*, of course, is similar in this regard. Again, for studies on reproductive technology, the straight cervix of *C. aethiops* is much easier to work with than the tortuous cervix of most macaque species.
4. Animals that can be handled and/or manipulated safely (e.g., for cognitive or behavioral testing). Those with enzootic virus B are awkward for such uses. The otherwise aberrant New World marmosets and squirrels may be excellent, due to their small size and temperamental malleability.

5. Primates that need no qualification other than to be as close to the human as possible in regard to immune mechanisms, pharmacokinetics, physiology, and so forth. For such use as nonspecific primates, examples of the criteria should be size, convenience, safety, price, and status of endangerment. The existence of relevant normative databases is also desirable.

These diverse needs are typically met by a single species the institution or investigator has chosen based usually on history, habit, chance availability, or housing space available, rather than on any of the rational criteria outlined above. The majority of animals used are the following: Asian macaques—mulatta, fascicularis, and nemestrina; African *C. aethiops* and *Papio* and New World *Saimiri* and *Saguinus*. I personally have worked with *Saimiri*, *Saguinus*, *Cebus*, *Ateles*, *M. mulatta*, *M. arctoides*, *Papio*, *Pan*, *Chlorocebus* (*Cercopithecus*), and *Homo*. My own favorite primate is *M. arctoides*, but for the past 33 years I have concentrated on *C. aethiops*, SK, which has several features that commend its use as a complement to the more extensively used macaca in biomedical research.

The Caribbean islands of St. Kitts and Nevis are, in effect, closed breeding colonies that house approximately 40,000 to 50,000 *C. aethiops sabaeus* in a tropical ecology with no natural predators. It is 300 generations beyond a founding stock of approximately 1000 West African immigrants. Biochemical and molecular analyses document the genetic diversity of the population. Census data suggest that at least 5000 individuals can be harvested per annum without affecting population viability or diversity in any manner. In addition, we maintain a breeding colony of about 1000 animals, primarily for our own scientific work and that of collaborators; but this number also provides a base for developing cross-species comparative data (e.g., on pharmacokinetics and reproductive endocrinology). In addition, our breeding program can selectively target the expansion of rare naturally occurring phenotypes and the development of disease-appropriate experimental phenotypes. With 30 years of experience in establishing practical husbandry and breeding programs for 1000 to 1500 animals in addition to an ongoing research and training program with emphasis on developmental biology, the establishment of normative databases, and quantitative measures of behavior change, we can complement the abundant supply of animals with professional screening selection and advice as to handling.

The St. Kitts vervet was removed from West Africa before 1700 by early French settlers to the New World. It thus escaped the major pathogens that infested contemporary African populations. In the Indies, it evolved in a predator-free environment to become the leading agricultural predator and threat to economic self-sufficiency.

This specific pathogen-free, nonendangered, readily available Old World primate has numerous immediately attractive features. It is:

1. B virus free. This macaque enzootic pathogen is instantly fatal to the vervet as it is to the human. It has been suggested that keeping a vervet in a laboratory colony of rhesus would serve as a "canary" to detect an outbreak of immunosuppression.
2. Nonendangered. Nowhere in its range is it endangered. It is the weed monkey of Africa, flourishing in most econiches. In the Caribbean, it is a significant crop predator and general pest.
3. Relatively small and temperamentally tractable, unlike rhesus (e.g., adult male maximum weight = 7 kg).
4. Also free of other pathogens. For example, there is no evidence of filovirus, SIV, or STLV, nor evidence of yellow fever, malaria, tuberculosis, yaws, or schistosomiasis.
5. Evolutionarily closer to the human, as is the baboon, than the Asian macaques. As a consequence, examples of methods in which most human reagents can be used in *C. aethiops* are PCR, SNP analysis, receptor binding, and cytokine measurement.
6. Four hours' flight from the United States and 6 hours' flight from Europe.
7. A candidate for on-site facilities for scientific preparation, quarantine, sampling, pretreatment, and so forth.
8. Highly cost effective.
9. A candidate for several spontaneous models of human disorder that have been identified to date and several others that have been readily induced (Table 1).
10. Relatively easy to breed in captivity, providing opportunities for genetic control, production of mother-fetus pairs for behavioral teratology, and infants for developmental studies and testing (Table 2).

TABLE 1 Models of Human Condition[a]

Spontaneous	Induced
• Hypertension	• Parkinson's disease
• Metabolic syndrome (X)	• Multi-infarct dementia
• Polycystic ovarian disease	• Allotransplant GvH syndrome
• Alcohol abuse and fetal alcohol syndrome	• Xenotransplant GvH
• Anxiety disorders including panic disorder	• Estrogen-induced uterine CA
• Mother-fetus dyads (e.g., for vaccine teratology)	

[a]See also Palmour et al. 1997. Am J Hum Genet 61: 481-488.

TABLE 2 Reproductive Characteristics

Sexual maturity: female, age 3; male, age 5-6
Menses: 28-day cycles during breeding season (October-March) in natural habitat. Anestrus cycles March-October, if not pregnant. Male testis in apparent regression during this period
Pregnancy: 6 months. Weaning by age 6 months. Twins about 1:88 (like humans)
Female fertility throughout life cycle but irregular pregnancy after age 20
Male fertility throughout life cycle

The Federation of St. Kitts and Nevis is an independent nation and a member of the United Nations, the Organization of American States, the British Commonwealth, and other regional and international bodies. It is signatory to the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) agreement and other relevant international agreements pursuant to such concerns as animal health and transportation. The indigenous primate population is designated a national resource, and the export is controlled by the Ministry of Agriculture.

The Behavioral Sciences Foundation is a not-for-profit research foundation, incorporated in Delaware and also established under the laws of St. Kitts and Nevis in 1968. It maintains laboratory and breeding facilities at the Estridge Estate on St. Kitts and has an affiliated institutional review board established under the principles of the Canadian Council on Animal Care.

Other information about the St. Kitts vervet is available at our academic website at http://www.crcmgh.com/carib or by mail. Please contact fervin@caribsurf.com for further information.

Session 1: Panel Discussion

Participants:
Christian R. Abee—Session Chair, University of South Alabama, USA
Mario J. Baudoin—Ministry of Sustainable Development and Planning, Bolivia
Mukesh Kumar Chalise—Natural History Society of Nepal, Nepal
Frank Ervin—McGill University, Canada
Jason M. Mwenda—Institute of Primate Research, Kenya
A. Jagannaha Rao—Indian Institute of Science, India
Jurgen Seier—Medical Research Council, South Africa
Mary Ann Stanley—Bioculture Mauritius Ltd., Mauritius

QUESTIONS AND ANSWERS

DR. ABEE (Christian R. Abee, University of South Alabama): Do you see a problem between conservation and providing animals for biomedical research? Is biomedical research likely to have an impact on indigenous populations worldwide?

DR. SEIER (Jurgen Seier, Medical Research Council): It is not a problem from a South African perspective, in terms of baboons and the vervet monkeys. I think the numbers are very stable and common in many areas. There are many more animals destroyed as agricultural pests.

DR. ABEE: Do you believe that each of your countries is sensitive to the issue of conservation with respect to primates?

DR. ERVIN (Frank Ervin, McGill University): Yes. We have, for example, set up sanctuaries, which are tourist attractions. Tourists love to look at monkeys; they are cute. We put the monkeys where the tourists are and took them away from where the agriculture is. We have made the monkey a stamp feature so there is a series of stamps on the St. Kitts vervet. There is a general array of procedures. We give lectures in the school system so there are ways of making this an important part of the culture. At the same time, it is one that has to be brought under control. On our island, they are like rats in Harlem. They really are not cute.

DR. BAUDOIN (Mario Baudoin, Ministry of Sustainable Development and Planning): There is also a positive relationship between use and conservation that is very often forgotten. If you can give value to an ecosystem by using some of its components, you contribute not only to the conservation of those components but also to those things that will never have economic value. One major difference is between the existences of a wild population versus the use of captive breeding, which I do not think are complementary. They are not mutually exclusive.

DR. VANDEBERG (John VandeBerg, Southwest Foundation for Biomedical Research): First, I would like to congratulate the panel on a superb job. You answered all of the questions that we asked you to answer, and I really appreciate the care that you invested in developing your presentations, which were all very clear and very concise.

Second, I want to ask the representatives of the countries that have banned the export of primates about the rationale of their governments. That is, clearly there is a need for monkeys from some of these countries today—the baboons and vervets—and there may well be the need for the introduction of more monkeys from Africa in the future. We have heard about the animals being poisoned and shot as pests, so I would like to understand why the governments are against the exportation of these animals for research.

DR. ABEE: Could I suggest that Dr. Rao speak first?

DR. RAO (A. Jagannadha Rao, India Institute of Science): I think one of the major problems is that all active export will probably not be restricted, which will contribute to their decline. In India, this situation started because there was no distinction in terms of animals being pests, being poisoned, or being killed. Actually, in fact, as I quoted in one of the presentations, there are now more of them than before. Still, they are very much revered and they are not harmed. Their export in large numbers caused their number to decline, caused the rhesus to be considered only in terms of conservation, and caused the export to be banned. Actually, the current numbers are quite large and they can be used differently, with very judicious use. However, now we have more regulatory problems rather than conservation problems.

DR. MWENDA (Jason Mwenda, Institute of Primate Research, Kenya): To clarify, the Kenyan government banned the export of primates for reasons relating to the humane handling of the animals. Commercial handlers had been selling the primates under the lead of the experienced. There was an outcry of animal rights because the commercial exporters of primates were not handling the animals humanely. The second reason was to encourage research to be conducted in the country. The government believed that having research done in Kenya rather than exporting the primates would contribute more to technology transfer.

DR. BAUDOIN: I was involved with drafting the 1987 disposition that banned wildlife trade. The situation was totally different from what it is now. There was very, very heavy trading in cats, for example. We had never had very strong national law enforcement because it is a huge country; however, the situation has changed now. The ban authorizes use on a species-by-species basis if enough information exists to warrant a decision. It is possible to develop the studies and study densities and then to propose the use.

I think the problem now has moved to a different arena. The animal rights discussion has biologists scared to approve those studies that would say, "yes, go ahead and use." So it has become a little more difficult, but I think it could be done.

DR. MCGREAL (Shirley McGreal, International Primate Protection League): I have a question for Dr. Rao and Dr. Chalise. As you know, India banned export of monkeys because of concern over the end use in military experimentation, such as radiation and bio-warfare, which is increasing in our country now but was against the Hindu tradition. Do you believe now, Dr. Chalise, that the Hindu tradition is not strong in Nepal and that Nepal would tolerate exports? My question for Dr. Rao is whether India still maintains a concern about end use in military experimentation on monkeys?

DR. CHALISE (Mukesh Kumar Chalise, Natural History Society of Nepal): Our concern is in the biomedical research, which the Natural Society has proposed and for which we are in the process of requesting approval. The proposal consists of four major points. Biomedical research is one of the components, which includes the conservation research and training program. We are specifically writing proposals that will not disturb the wild population, and we will use some of the animals to produce the offspring—only the offspring that we are utilizing. We have also assured the government that we will return the animals to the wild population. In addition, we will collect those required individuals only from the problematic areas.

As far as the Hindu concern, it depends on the committee. Nepalese comprise mostly Hindus; however, there are so many different groups

(we say "class systems"), and only very traditional Hindus believe such matters are religious. I think that if we are honest in our writing and our actions, there will be no problem with the people's religious belief.

Furthermore, it will help the Nepalese people and export to develop conservation. I am hoping to collect monkeys from the problematic areas and put them in the hands of the Nepalese government school of the conservation. Currently, there is a conflict of ideas between the government, conservation enthusiasts, and the ideas and interests of the people regarding the perfect areas. People sometimes ask whether the government has a greater need for its wild animals or its people? We must think about the conservation issues because people become angry at the idea of conserving the monkey. It is very different from India.

DR. SEIER: I would like to comment on the question regarding transportation and government policies. In the case of South Africa, it was not at all a government policy. It was also not an animal welfare consideration. South African Airways is a government organization in a sense, but they do make their own business decisions. Essentially, they are autonomous from the government in business. Their most lucrative routes are to Europe, where they do not wish to be targeted by animal rights people, and political people, by transferring primates. I do know and have copies of communication from UK animal rights people to South African Airways in which they were asked to state whether they are in principle willing to or currently transporting primates for biomedical research. I believe those groups write to every airline. That is the main reason they are not going to sacrifice their most lucrative business by taking a stand on transporting primates for biomedical research. We are talking not about trading but about even transfers of small numbers to other research organizations that need vervet monkeys. We have addressed this issue occasionally from Europe and the United States, that they are needed because they are free of SIV and other pathogens. This reason, not so much a government decision, formed the basis.

DR. RAO: As for the question about the animal activists, if you can strike a dialogue with them, I do not think India will see a problem with the society in terms of using animals for biomedical research. Some of you might have seen recent newspaper accounts that the rhesus is very afraid of the langur. There is a need to see that the animals are removed from these areas and are repopulated elsewhere so they are not a menace. The major problem is to convince these agencies that they can relocate there and will be judiciously used.

Unfortunately, under our current situation, no dialogue is possible. Otherwise, it would be possible to use enough rhesus for biomedical research or to allow other agencies from other countries to obtain them for use in separate established laboratories so that eventually the restric-

tion could be lifted and they could be exported. I would like you to be aware that we are attempting to handle this major problem in our available animals.

DR. ABEE: I was struck by Dr. Baudoin's comment about creating a sense of value among the indigenous people. It seems to me that it is difficult to communicate the concept of conservation to people who are living day to day, trying to find enough food to survive. I believe this concept of creating value for the forest to conserve the fauna and flora is very important. I wonder if you have anything further you would like to say about that?

DR. BAUDOIN: I think the problem is very simple. If you have a person in front of a tree, he must make a decision without considering 100% of the biodiversity in the forest. However, it is certainly much better than total replacement of the ecosystem by a much simpler system. Unless we can give value (in the sense of economic value) to some of the components of the forest, the person does not have much of a choice.

PARTICIPANT: I understand there was a great amount of concern over the high price of certain kinds of tropical butterflies and that the Japanese and the Germans, in particular, who were collecting these butterflies, were suspected of depleting the national population of these butterflies. Some studies that were done to look at this found that the net effect of all this butterfly collection was actually extremely positive. It reduced the rate of deforestation. If you think about it, 9 months of hard agricultural/burn labor or a couple of weeks in the forest with a butterfly net yields the same net income for the people actually doing the catching. Obviously, insects reproduce much faster than monkeys; however, there was no detectable negative impact except in a couple of very localized areas on the populations of these animals. So I think that one way of approaching this concept of sustainable extract is that in fact, you are preserving the ecosystem by enhancing the value that exists to the people that live there.

MR. BAULU (Jean Baulu, Barbados Primate Research Center and Wildlife Reserve): I would like to comment on the problems Dr. Seier described with airline transportation and so forth. We in Barbados produce at least 800 monkeys a year on average and 80% of the world's polio vaccine through the monkeys' kidneys that we send or the monkeys themselves. We had a problem with Air Canada, which just decided arbitrarily to ban the shipping of monkeys. We took them to court and after 3 years, we won. So when you really care about what you are doing and you have a good reason, you take them to court—it is as simple as that. I know that everybody here has problems with airlines. I believe we need to form a coalition of some kind and bring them one by one to court—because we will win.

DR. ROSENTHAL (Josh Rosenthal, NIH Fogarty Center): We have been working for several years to develop systems of sustainable use of biodiversity, in our case, plants and microorganisms for pharmaceuticals development and benefit sharing schemes to provide incentives for conservation. With success in some areas and less success in others, part of the key to those kinds of schemes is having mechanisms that relate relatively near term income to the people who are truly likely to be users of the resources. This task would be quite complicated, particularly with not very highly structured societies in a difficult regulatory environment; yet it is basically a good idea. One critical point that comes to my mind is that the situation with monkeys as an important pest in agricultural environments relates to a key information need. Very good documentation of those effects and an effort to get that message out to the public in a regular way would be one way of combating the very canalized interests of animal rights groups who then work against the airlines and other kinds of interests to promote use.

MR. GRIFFITHS (Owen L. Griffiths, Bioculture Ltd.): I would like to add something to this theme about biodiversity, primates, and conservation and to clarify Dr. Stanley's allusion. In Mauritius, for every monkey that is exported, the government of Mauritius collects $50 US. As you can see from Dr. Stanley's figures, about 5000 monkeys are exported by our group of companies, totaling a quarter of a million US dollars every year and going straight to the national parks conservation fund that runs conservation projects in Mauritius and includes weeding native forests, getting rid of exotics, and building predator fences to keep out (so far) pigs and deer. It is a fundamental part of the conservation program in Mauritius. It is always very frustrating for us that although animal rights people, specifically from the UK, claim to be conservationists and say, "ban the use of monkeys, ban the export of monkeys from Mauritius"; yet this quarter of a million dollars a year is simply fundamental to conservation in Mauritius. Clearly there is no other equivalent source of funds available.

DR. ERVIN: To reinforce Mr. Griffiths' point, I think that nearly everyone who lives in this situation is aware of it, and it is similar on St. Kitts. For each exported monkey, a levy is paid into a special fund within the Department of Agriculture that goes to conservation, research, and education. Each farmer from whose farm a trapper collects monkeys not only has his crop protected but also receives a percentage of the trapper's fee. So we now turn this country's worse predator into a cash crop from which we are selling the weeds—and at a good price so that everyone at every level can understand and benefit: immediately in terms of cash, and abstractly in terms of knowing, because of work in the schools and the

newspapers that this monkey is making possible our understanding of a major brain disease or hypertension.

DR. ABEE: And I think on the side of the biomedical research community, that the biomedical research community is very pleased to hear that this possibility will result from the money being generated from this.

DR. LYONS (Leslie A. Lyons, California National Primate Research Center): I think Dr. Mwenda made a very important point that some of the exportations have stopped because of a desire to keep research in the countries where the animals are. Does the panel think that perhaps if the NIH or the US government invested more in training for the countries, that we could open up exportations a little bit more as a give and take to help get animals back and forth?

DR. RAO: I think there is one step toward using the situation. There is a strong feeling by conservation biologists as well as animal activists that the animals are being used as a cash crop, proving that the best thing is to develop a system of centers with organizational help, such as what NCRR attempted in Bombay. Once people understand how useful they are, they will probably support the decisions, and exporting will be possible.

DR. LYONS: Additionally, perhaps while we are waiting for the possible change of exportation laws, we can change the thinking of our researchers. How would we propose to have more researchers think more about vervets than macaques?

DR. ERVIN: They need to be better educated.

DR. ABEE: Actually I thought Dr. Ervin did a very good job of that already. I would probably buy life insurance from him also.

I would like to thank all of our speakers for coming so far to share their thoughts with us. I think this session has been excellent. Thank you.

Session 2

Conservation and Supply, Part 2

Nonhuman Primates in Preclinical Research: The EU Situation

Gerhard Hunsmann, MD

In March 2002, a survey was conducted among 34 institutions involved in nonhuman primate (NHP) research located in eight European Union (EU) countries and Switzerland to identify the numbers of NHPs imported, bred, and used for biomedical research. The questionnaire (Table 1) was sent to the scientists responsible for NHP studies in public and industrial facilities. The response rate in the public sector was 79% (15/19) and from industry, 67% (10/15). While among institutes in the smaller countries using fewer NHPs in biomedical research the response to the survey was 100% (except Belgium), the return from NHP users in the larger countries with larger numbers of NHPs used for preclinical work in academia and industry ranged between 61 and 83%.

BREEDING OF NHPS IN EU COUNTRIES

The overall number of NHPs bred for biomedical research in European institutions in 2001 was 1120: 518 common marmosets (*Callithrix jacchus*); 564 animals of the two macaque species—rhesus monkey (*Macaca mulatta*) (n = 311) and long-tailed or crab-eating monkey (cynomolgus, *Macaca fascicularis*) (n = 253). Smaller numbers were also bred of the common squirrel monkey (*Saimiri sciureus*) (n = 19) and the cotton-top tama-

German Primate Center, Goettingen, Germany

TABLE 1 Questions to the Nonhuman Primate (NHP) Users

Do you breed NHPs (species, annual production)?
Do you produce/require specific pathogen-free (SPF) and/or genetically characterized NHPs?
Do you import NHPs (species, number of animals per year)?
From which country do you import NHPs?
What type of work is conducted with these NHPs?
Which are the financial resources for this work?
Should the number of NHPs bred for biomedical research be increased in Europe?
Are you aware of any further NHP user not yet included in our mailing list?

rin (*Saguinus oedipus*) (n = 19). Although Germany breeds the majority of marmosets (n = 320), the United Kingdom produces most of the crab-eating monkeys (n = 200) (Figure 1). With respect to production and usage of common marmosets, Europe is self-sufficient.

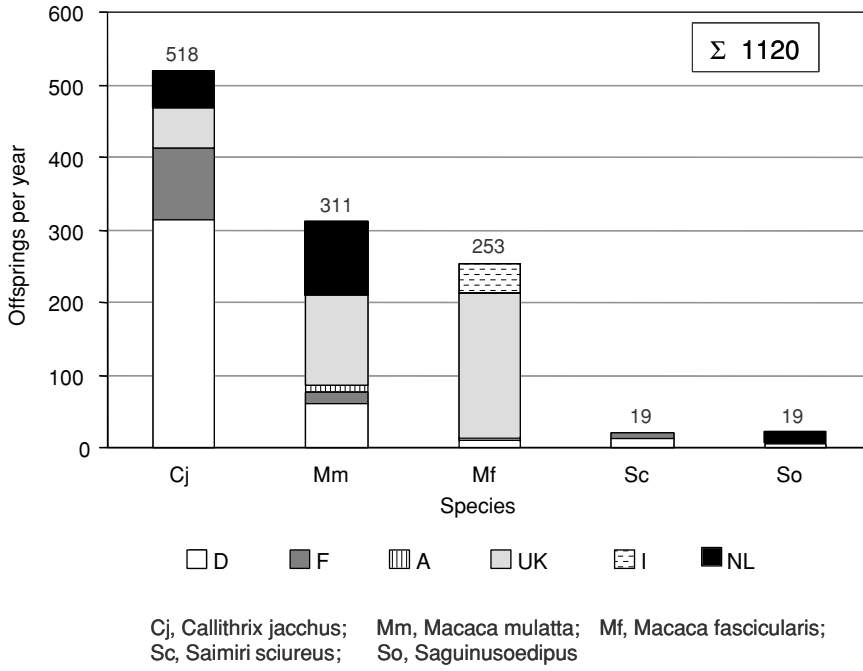


FIGURE 1 EU-bred NHPs. Species codes: *Cj, Callithrix jacchus* (common marmoset); *Mm, Macaca mulatta* (rhesus monkey); *Mf, Macaca fascicularis* (long-tailed or crab-eating monkey); *Sc, Saimiri sciureus* (common squirrel monkey); *So, Saguinus oedipus* (cotton-top tamarin). Country codes: D, Germany; F, France; A, Austria; UK, United Kingdom; I, Italy; NL, The Netherlands.

IMPORT OF NHPs

Last year, 1866 NHPs were imported into the EU belonging to the two macaque species (Figure 2a). A total of 74% originated from commercial breeders in China, 12.5% from Israel, 10% from Mauritius, 3% from La Réunion, and 0.5% from the United States (Figure 2b). These numbers are certainly slightly underestimated since the return of the questionnaire was not 100%, and one institute's response did not communicate the number of animals used annually. The percentage of imported NHPs differs significantly in individual European countries from 100 to 36%. The overall number of NHPs currently imported into the EU slightly more than 2000 annually. This total is up to 70% of the NHPs used for biomedical research in both academic and industrial institutions; 74% of macaques imported originate from a single country.

THE NHP BREEDING GAP

Figure 3 indicates the difference in numbers of NHPs used and bred in each individual country. Although some countries depend totally on NHP importation, others have substantial breeding facilities. The overall number of imported NHPs was about 2000 in the year 2001. Consequently, probably up to 70% of NHPs required for biomedical research in the EU countries and Switzerland must be imported.

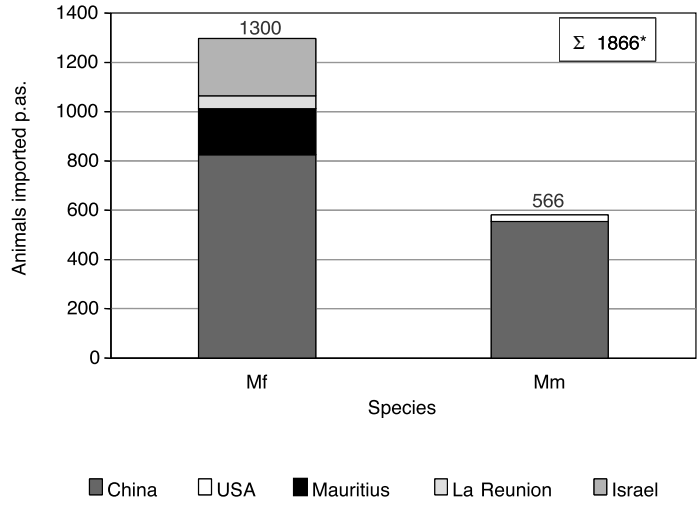
SPECIFIC REQUIREMENTS FOR NHPs

There is an increasing need for specific pathogen-free NHPs, as well as for those typed for major histocompatibility complex (MHC), which have a known pedigree or specific blood groups. More than half of the institutes replying to the questionnaire would require such animals, but sufficient numbers are unavailable as yet. Only four breeding colonies producing a limited number of animals free of at least some unwanted pathogens exist in Europe. Likewise, only three institutes are able to provide a few MHC-typed macaques.

EXPERIMENTAL PROTOCOLS AND FUNDING

In institutions of the pharmaceutical industry, most animals are used for pharmacokinetics and toxicology studies. However, university laboratories and public research centers require NHPs for a broad spectrum of research in the fields of neurosciences, reproduction and fertility control, cardiovascular and metabolic research, gene therapy, infectious disease models and vaccine studies, and immunological studies on allo- and xenotransplantation, as well as on allergy and autoimmunity.

A. Annual import of NHPs by species



B. Origin of NHPs annually imported into Europe

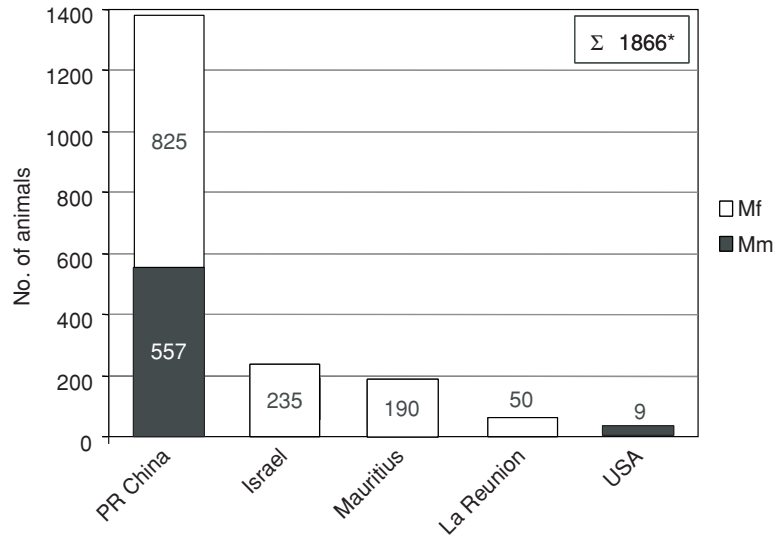


FIGURE 2 Importation of NHPs into Europe. (A and B) For country and species codes, see Figure 1 legend. (B) white column indicates *Mf;* and black column *Mm*. *These census numbers are incomplete due to missing information from one responder. The percentage refers to the overall number of imported NHPs.

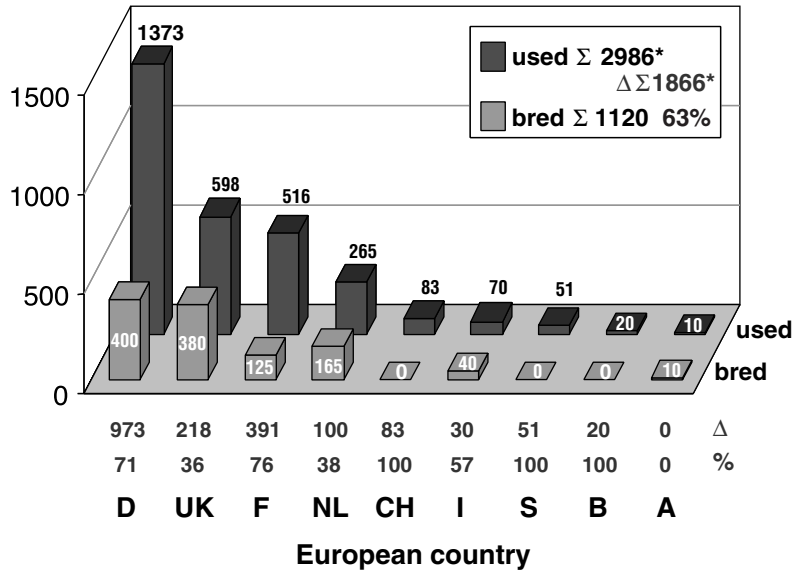


FIGURE 3 Annual number of NHPs bred and required. For country codes, see Figure 1 legend; CH, Switzerland; S, Sweden; B, Belgium. Δ indicates the number of NHPs imported by each country, the percentage gives the rate of imported animals. *Refer to Figure 2 legend. % is the percentage of animals that are imported.

Industrial studies are funded through the respective pharmaceutical companies. However, a substantial fraction of NHPs kept in Europe are being maintained through public resources originating from ministries of health and research and defense, as well as public funding agencies such as the Max-Planck-Gesellschaft, Deutsche Forschungsgemeinschaft, Agence Nationale de Recherche sur le SIDA, Medical Research Council, and the Wellcome Trust.

SUMMARY AND CONCLUSION

The overall response rate to the questionnaire was 73% (25/34), with an unexplained country-specific variability of 25 to 100%. Academic institutions responded more frequently (79%) than industrial facilities (67%).

The number of NHPs used for biomedical research in the European countries surveyed adds up to slightly more than 3000. Approximately two thirds of those must be imported from breeding facilities outside Europe. Of these animals, 70% originate from the Peoples' Republic of China. Most of the researchers contacted believe that NHP breeding

within Europe should be increased to reduce the overall number of imported primates and the dependence on a single importing country. Moreover, NHPs with certain microbiological and genetic specifications are required in increasing numbers.

ACKNOWLEDGMENT

I am indebted to colleagues of 25 institutions from academia and industry in nine European countries for providing critical information to this survey.

Providing Investigators and Vaccine Producers with Laboratory Primates in the Russian Federation

Boris A. Lapin, PhD, MD

The use of laboratory primates in Russia has decreased sharply during the last 10 to 12 years due to economic conditions, the limited possibility of purchasing monkeys in their natural habitat, the difficulties of their transportation, and, quite often, complex veterinary conditions in the countries where the monkeys naturally live, which hampers obtaining veterinary permit for importation. Today the number of countries exporting monkeys from their natural habitat has also decreased sharply. The variety of species offered by the exporters is as a rule limited to *Papio anubis* and African green monkeys. The above-mentioned difficulties are aggravated by the refusal of many air companies to ship monkeys in passenger flights, necessitating charter flights and ordering more animals than are needed at the time, both of which increase expenses.

The Russian Federation provisions for fauna do not include the primate species. The supply of primates for experiments has been covered either by importing primates from their natural habitat or by breeding them in the pedigree monkey colony of the Sukhumi Institute of Experimental Pathology and Therapy of the USSR Academy of Medical Sciences (IEPaT AMS). The main colony and several of its branches in the vicinity of Sukhumi numbered about 7500 monkeys, mainly *Papio hamadryas* and

Institute of Medical Primatology of the Russian Academy of Medical Sciences, Sochi-Adler, Russia

Macaca mulatta of Indian origin. In addition, after the ban of export macaques from India, a small number of macaques of Vietnamese and Chinese origin were imported to increase the monkey livestock. Besides rhesus monkeys, the Sukhumi monkey colony maintained *M. fascicularis* and small groups of *M. nemestrina* and *M. arctoides*. *P. hamadryas* numbered 2000 animals. There were also small numbers of *Papio anubis*, hybrids *P. anubis* × *P. hamadryas*, and African green monkeys.

As mentioned above, the total number of monkeys in the Sukhumi monkey colony was 7500 and the annual number of newborns was between 800 and 900. Monkey harems were housed in special monkey houses in large cages with floor areas of 10 to 12 m^2 and large open compounds with floor areas of several hundred square meters. Many monkeys were kept in mountains and forest reserves in conditions similar to those existing in their natural habitat. To train monkeys not to go far away, they were fed every day in a definite place at a definite time. It was very important to feed them when it was snowing in the mountains. Despite the considerable decrease in the surrounding temperature, the monkeys acclimated very well to the cold weather; they did not catch cold, and not a single case of death from supercooling was registered. Each monkey from the colony or game reserve has its life history documented with information about how the animal moves from area to area, from cage to another cage, or to the open compound; the laboratory analyses (once a year); the pregnancy and delivery of babies, and so forth.

The number of monkeys including newborns in the Sukhumi monkey colony has met the need of all Russian institutions using monkeys in experiments except for the polio vaccine producers. It was necessary for them to import monkeys from India, Vietnam, or China. At first, mainly *M. mulatta* were imported; however, due to the subsequent high rate of macaques infected with SV-40, African green monkeys were substituted according to advice from the World Health Organization. I did not agree with this decision because African green monkeys are often infected with STLV-1 and EBV-like viruses. However, it was necessary to import about 3000 of African green monkeys to obtain cell cultures and control residual neurovirulence.

The method of growing vaccinia virus has now been improved, and the bulk of the monkeys have been used to control the residual neurovirulence of the polio vaccine. Taking into consideration the considerable reduction of primates in their natural habitat, which I believe will affect African green monkeys in the near future, the Commission on Medicobiological investigations on monkeys under the RAMS Presidium has now recommended that polio vaccine producers develop measures for sharply reducing their use of African green monkeys in polio vaccine production.

After the military conflict between Abkhazia and Georgia had arisen, it became impossible to continue working in the Sukhumi Institute, and the main staff had to move to Sochi-Adler. There, on the basis of an existing branch of IEPaT AMS, a new Institute of Medical Primatology was founded with a large monkey colony affiliated with the Russian Academy of Medical Sciences (IMP RAMS). The Adler monkey colony currently has 2500 monkeys, including 1,000 *M. mulatta*, 600 *M. fascicularis*, 500 *P. hamadryas*, 100 *P. anubis*, 100 African green monkeys, and a few animals of other species. Every year the Adler monkey colony is reinforced with 500 newborns of different species. The IMP RAMS in Adler carries out its own wide research program, and the Institute also provides visiting researchers from the Institutes of RAMS and the Health Ministry of the Russian Federation with the opportunity to carry out experiments on monkeys. Some researchers perform their investigations as collaborators according to the signed agreement between IMP RAMS and the other Institutes concerning testing of vaccines and biopreparations.

To meet the national need for monkeys independently from importation, IMP RAMS plans to increase the livestock of monkeys up to a total of 4000. To fulfill this plan, we intend to construct more monkey houses and corrals during the next 3 years. Only the Institute of Poliomyelitis and Virus Encephalitis is still an exception and must still import African green monkeys annually to produce polio vaccine and to test residual neurovirulence. Taking into consideration the above-mentioned situation that limits the importation of monkeys, it becomes clear that we can rely only on monkeys bred in colonies in captivity. This approach ensures that we will diminish the risk of infection imported from the monkeys' natural habitat, which can be dangerous for the monkey colonies, the monkey handlers, and the experimenters alike.

One can predict that in the near future, the only way to obtain monkeys for experiments will be to develop monkey colonies and breed them in captivity. The sooner we use monkeys bred in colonies, the more effectively we will be able to protect and save endangered species of monkeys, even though the main reason for reducing the natural monkey population is the economic activity of humans.

Nonhuman Primate Resource Needs: A Moving Target

Jerry Robinson, PhD, and Greg Beattie, MSc, D.A.B.T.†*

Thank you, Mr. Chairman. I also wish to thank the organizing committee for convening such an extremely important meeting to address the growing needs for nonhuman primate resources for biomedical research. This is a joint presentation by Dr. Greg Beattie of the Sierra Biomedical Research Division of Charles River Laboratories, Inc., and myself. The reason for our collaboration is that Dr. Beattie is involved in the commercial enterprise of providing nonhuman primates for biomedical research. I, however, am more involved with National Institutes of Health (NIH) grants that support biomedical research efforts predominately at academic institutions. It is hoped that the two of us will cover two distinct aspects of nonhuman primate research resources.

My major role at the NIH National Center for Research Resources (NCRR) is the administrative oversight of our National (formerly Regional) Primate Research Centers program (NPRC). In addition to the eight National Centers across the country, NCRR supports additional nonhuman primate resource centers at other sites in the country. I am also

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†Vice President, Operations, Sierra Biomedical Division, Charles River Laboratories, Inc., Sparks, NV

the program administrator of the NCRR grants that support these, thus, I am fairly familiar with the nonhuman primate resources that are supported by NCRR. In short, NCRR:

- Serves as a catalyst for discovery for NIH-supported investigators throughout the nation;
- Creates, develops, and provides a comprehensive range of human, animal, technological, and other resources to enable biomedical research advances; and
- Seeks scientific knowledge that will lead to better health and reduced illness and disability for our nation's citizens.

NCRR is rather unique among the Institutes that make up the NIH. NCRR is *not* a categorical disease institute like Cancer, Infectious Diseases, or Mental Health. However, NCRR, through grants, supports the basic infrastructures on which the biomedical research community depends to accomplish their biomedical research goals. We believe that we are the catalyst for biomedical research discoveries.

One of the major programs within NCRR is the NPRC Program. Each NPRC is affiliated with a major academic institution. The NCRR grants that support these Centers provide funding to support the basic infrastructure, which includes specialized animal housing facilities, animal care staff, veterinary care, and clinical laboratories, as well as the other specialized equipment and personnel necessary for the conduct of biomedical research. These eight Centers are a unique network that provides access to more than 20,000 nonhuman primates and provides the infrastructure support for more than 1200 scientists. Obviously, these National Resource Centers play a critical role in the conduct of biomedical research using nonhuman primate models.

One of the key questions that speakers were charged with addressing was to identify "the species and numbers of monkeys that are maintained in your country or region for biomedical research." NCRR recently completed a survey of 1999 NIH grantees who use nonhuman primates for research. Approximately 13,000 nonhuman primates were used in 1999 by NIH grantees. Of those 13,000 animals, nearly two thirds were rhesus macaques.

According to the US Department of Agriculture (USDA) annual survey of the number of animals used in 2000 (2001 figures are not yet available), more than 57,000 nonhuman primates were used in biomedical research that year. Unfortunately, the USDA does not report a breakdown by species, so we have no way of knowing which species are utilized in the greatest numbers. However, macaques, especially rhesus, are the most likely used as a research animal model.

So where do we get animals for biomedical research? Dr. Doug Bowden, Washington NPRC, gave a talk in Japan earlier this year and has shared some of the data he compiled. As can be seen in Table 1, overseas breeding colonies provide the majority of our nonhuman primate resource needs. US primate centers and other primate breeding facilities do provide significant numbers of animals. We are also aware of US commercial breeding operations, but we do not know how many animals are produced or the numbers used in the private sector.

Figure 1 provides a breakdown by species of the animals imported from 1995 to 2000. The long-tailed macaque was imported to the greatest extent. Although importation of rhesus is second, the majority of these macaques are imported mainly from China. Exportation of rhesus monkeys from India has been banned since the mid-1970s. Later in the program, Mr. Tom DeMarcus from the CDC will perhaps provide us with more up-to-date statistics in his presentation on the importation of nonhuman primates.

In Figure 2, the major countries appear from which nonhuman primates are derived. Again, the majority of the animals are long-tailed macaques and more than half are derived from Indonesia and Mauritius.

As I mentioned earlier, NCRR has a major program that supports the National Primate Research Centers. As can be seen in Figure 3, the majority of these Centers are located in the south and on the west coast. NCRR, through Center base grants, provides support for specialized facilities, personnel, equipment, and nonhuman primate resources for the conduct of biomedical research. These Centers provide infrastructure support for several hundred scientists doing research in neurosciences, infectious diseases, cardiovascular diseases, and other related human health problems that require nonhuman primates as the animal model. Such research efforts are supported by more than 500 NIH grants as well as other funding sources.

The rhesus macaque is the predominant species maintained at the National Primate Research Centers (Figure 4). However, significant numbers of baboons and long-tailed and pig-tailed macaques can be found at half of these Centers as well. Some of the Centers do maintain colonies of

TABLE 1 Sources of US Research Primates: 2002

Overseas Breeding Colonies	11,400
US Primate Center Breeding Colonies	2,000
Other Research Breeding Colonies	4,000
Primate Supply Info Clearinghouse	3,000
US Commercial Breeding Colonies	???

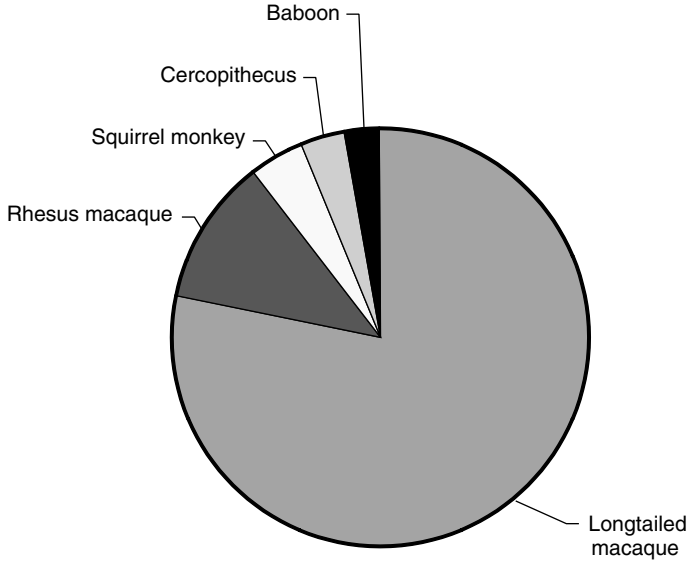

FIGURE 1 US primate imports by species (>1%): 1995-2000.

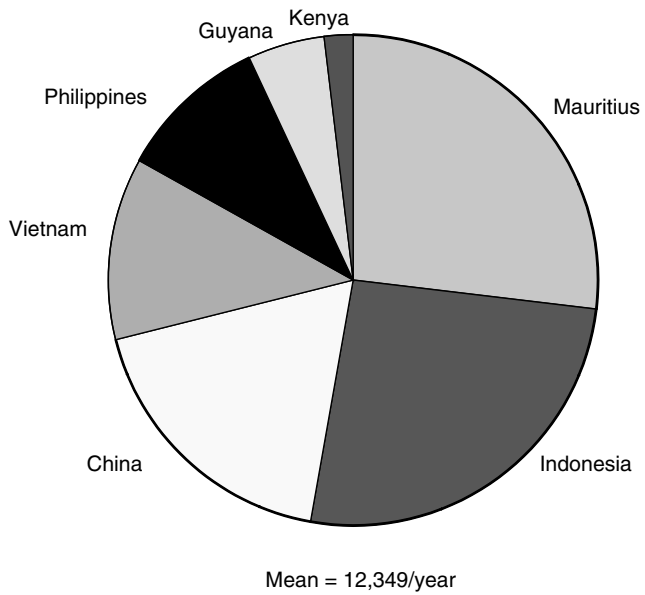

FIGURE 2 Countries of origin of US primate imports (>1%): 1996-1999.

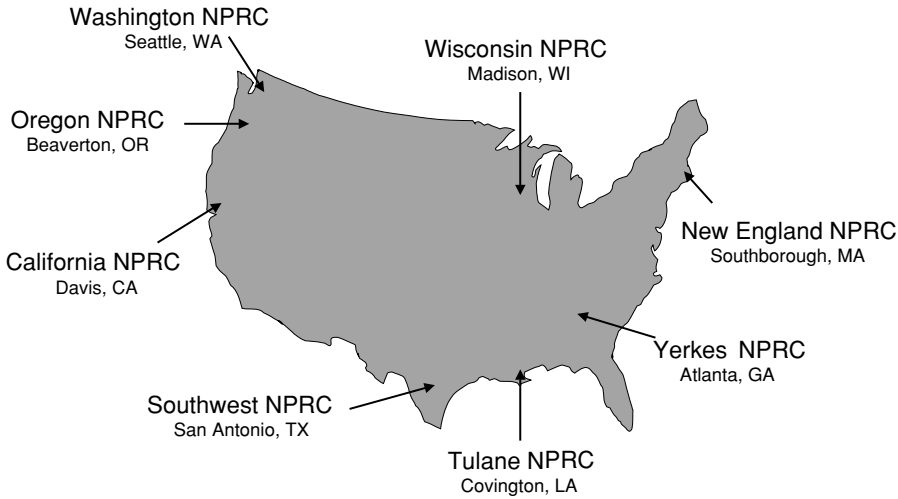

FIGURE 3 National Primate Research Centers.

Rhesus macaques (*Macaca mulatta)*		
8 NPRC s		**13,547**
Pigtailed macaques *(M. nemistrina)*		
4 NPRC s		**1,442**
Long-tailed macaques *(M. fascicularis)*		
5 NPRC s	**996**	
Indonesian Breeding Facility	**~2,000**	**2,996 +**
Japanese macaques *(M. fuscata)*		
1 NPRC		**236**
Baboons *(Papio sp.)*		
5 NPRC s		**4,103**

FIGURE 4 NCRR-supported nonhuman primate resources: Old World monkey census at NPRCs. NCRR, National Center for Research Resources; NPRC, National Primate Research Center.

new world species, marmosets, tamarins, and squirrel monkeys. In addition, NCRR supports the following: large colonies of rhesus macaques (n=2029) at the University of Puerto Rico's Caribbean Primate Research Center; a squirrel monkey colony (New World species; n=439) at the University of South Alabama; a baboon resource colony (n=167) at the University of Oklahoma; and two chimpanzee colonies located in Bastrop, Texas (n=180), and New Iberia, Louisiana (n=130). The squirrel monkey colony is our only Center dedicated solely to New World species. This animal model is excellent for malaria studies as well as other infectious diseases such as Chagas' disease. There are also increasing demands for baboons, particularly in the area of organ transplantation and diabetes. The chimpanzee colonies were established initially as potential models for AIDS research. Although the animals could be infected with HIV, they never developed the disease. These animals, however, are still good models for hepatitis and respiratory syncytial viral diseases.

For the rest of my talk, I wish to focus on the species that is in the greatest demand, the rhesus macaque. From the NCRR survey of 1999 NIH grantees, we learned that the rhesus macaque comprised approximately 65% of the nonhuman primates used for biomedical research. In addition, we learned from examining the animal census at the NPRCs that 60% of the animals being maintained at these Centers are rhesus.

So what are these animals being used for? One of the greatest demands these days is for AIDS research, particularly for testing potential vaccine candidates. Simian immunodeficiency virus infection in macaques almost exactly parallels HIV progression and pathogenesis in humans. At present, it is the only real animal model for studying this disease. The recent anthrax scare has created additional demands for the rhesus macaque. Initial testing of vaccines in the 1960s by the Department of Defense used rhesus as the animal model. To test new vaccines and therapies, they obviously want to use this species again.

There have also been significant developments in the neurosciences using nonhuman primates. There are nonhuman primate models for Parkinson's and Alzheimer's diseases. Scientists at the California NPRC have developed gene therapy techniques for inserting genes to produce nerve growth factor as a treatment method for overcoming neurological deficiencies in macaques. Similar treatments may be used in the treatment of Parkinson's. The nonhuman primate has also played a key role in the reproductive sciences: cloning, transgenic monkeys, and the establishment of embryonic stem cell lines. Rhesus macaques are also in demand for studies examining organ/tissue transplantation; they are the animal models used for kidney and pancreatic islet cells experimentation.

It is obvious that there is an ever-increasing need for nonhuman primates. To help meet the needs of the biomedical research community, the

NPRCs have increased their breeding colonies (Table 2). From 1996 to 2000, the number of animals in the breeding colonies has increased from 6200 to more than 7600. Although this increase is significant, not all of these animals are capable of reproducing at the present time. The rhesus female does not reach menarche until 2.5 to 3.0 years of age, and male puberty does not occur until 3.5 to 4.0 years of age. Thus, many of the animals in the breeding colonies are just getting to their reproductive age (Table 3).

Part of the problem is that the greatest demand is for animals 2 to 5 years of age. However, to build up the breeding, these are the animals, particularly the females, that are needed to increase the breeding stock (Table 4). Given the length of time needed for the monkeys to reach sexual maturity, we are looking at a 5 to 10 year period to increase production capabilities at the eight NPRCs significantly.

Other complicating factors that are putting additional strains on rhesus supplies come from the demands for specialized resource needs. The AIDS research community as well as those investigators looking at transplantation tolerance need animals free of specific pathogens. Animals not free of certain retroviruses can compromise the experimental findings. There is also a need for genetically defined animals; for example, certain types of AIDS research require animals with a known major histocompatibility complex. Such demands further limit availability of these precious resources.

Rhesus macaques of Chinese origin have become available and were thought to be a possible solution to the limited supply of rhesus. However, the different immunological and genetic makeup of these animals has led to some research complications. The pathogen status of some of these animals is also problematic. Nonetheless, these animals can be utilized in certain types of experimentation.

TABLE 2 National Primate Research Centers, Rhesus Macaque Colony Statistics

		Breeding Colony Statistics	
Year	Animal Numbers	No. Animals	% Total
1996	11,706	6,271	53.4
1997	11,641	5,580	50.3
1998	11,828	6,028	51.0
1999	12,546	6,023	48.3
2000	13,584	7,620	56.1

TABLE 3 Rhesus Demographics at 8 NPRCs: January 2001

Total Number of Animals:		13,584	
Number of Animals—Asian Indian Origin		12,038	88.7%
Number of Animals—Chinese or Hybrid		1,529	11.3%
Age Distribution:			
0-5 years of age	7,929	58.4%	
6-10 years of age	3,278	24.1%	
11-15 years of age	1,323	9.7%	
16-10 years of age	582	4.3%	
>20 years of age	482	3.5%	

Another problem confronting investigators is a limitation of specific types of housing. Many research protocols, particularly vaccine challenge studies that study infectious diseases, required specialized caging (biosafety level 2, 3, and even 4 in some cases). Although the NPRCs are trying to increase their holding facilities, their current capacity is very limited and is being utilized to the maximum. Studies of infectious organisms such as anthrax will place even greater demands on specialized housing needs.

So what is NCRR doing to try to help meet these needs? In September 2000, NCRR awarded five grants to establish rhesus macaque colonies that are free of specific pathogens (SPF colonies) and then added a sixth colony in 2001 (Table 5). These colonies were established in coordination with the Office of AIDS Research. Thus, the offspring generated will be dedicated to the AIDS research community. It is projected that these colonies will produce more than 2300 animals in 5 years.

Because the projected needs from the NCRR survey include more than 7000 animals and we are aware that an increasing number of vaccine candidates must be tested, NCRR reissued the announcement soliciting the establishment of additional colonies in 2002. It is anticipated that five

TABLE 4 National Primate Research Centers: Rhesus Monkey Statistics May 2001

Total Numbers			13,547
Asian Indian Origin	12,016	88.7%	
Chinese Origin	1,531	11.3%	
No. of Animals in Breeding Colonies		56.1%	7,604
No. of Females Capable of Conceiving			2,921
No. of Offspring Produced 2000			1,828

TABLE 5 NCRR-Supported SPF Rhesus Colonies

Site	Host Institution	5-year Projections
1. California NPRC	University of California-Davis	215
2. Caribbean PRC	University of Puerto Rico	350
3. New England NPRC	Harvard University	335
4. Oregon NPRC	Oregon Health Sciences University	700
5. Southwest NPRC	Southwest Foundation for Biomedical Research	365
6. Tulane NPRC	Tulane University	400
	Projected Totals	2,365

or six more SPF colonies will be supported to help meet these growing needs. Again, we are talking about a 5- to 10-year period to establish these colonies to the point where they begin producing significant numbers of monkeys.

Obviously, NCRR cannot provide all of the nonhuman primate resources needed by the biomedical research community. We are trying to facilitate importation of more macaques from international sources to help meet some of these needs. However, some investigators may have to turn to the private sector to meet their animal needs (Table 6). Alternatively, other investigators may want to consider other nonhuman primate species as alternative models for their studies of human diseases. Other nonhuman primate species such as the long-tailed macaque are more readily available.

TABLE 6 NPRC—Commercial Relationships

- Some NPRCs obtain up to 50% of their animals from commercial sources
- Research by companies can be accomplished at the NPRCs, but only accounts for 5%-20%;
  - NIH supports about 80% of biomedical research in the US
  - Most commercial use of primates is routine toxicology; not a common focus of university researchers' interest
  - NIH does not subsidize company research, so costs are high
  - Intellectual property considerations: universities encourage publication, which can be counterproductive for companies
- Most US pharmaceutical and biotech companies either have their own primate research facilities or contract the work to private research companies.

Center for the Breeding and Conservation of Primates of the Peruvian Primatology Project

Enrique Montoya, DVM

BACKGROUND

There is worldwide concern about preserving the environment and its natural resources since current and future generations have the right to enjoy an environment that is healthy, balanced, and suited for life, in harmony with the landscape and nature. Significant trends have had an impact on natural resources in the Peruvian Amazon. The 19th century and the first half of the 20th century were characterized by the exploitation of plant resources. Then in the 1960s, depredatory trade in wild fauna and their by-products proliferated, exporting live specimens such as mammals, psittacidae, and ornamental fish.

In 1974, Peru ratified the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and promulgated the Wild Flora and Fauna Act (decree law no. 21147), which regulated the hunting or capture of species of wild fauna and their use for scientific and cultural purposes. In 1972, the Study on Nonhuman Primate Populations in Peru was promoted, with the involvement of the Veterinary Institute of Tropical and High Altitude Research (IVITA) of the School of Veterinary Medicine of the San Marcos National University (UNMSM) and technical cooperation from the Pan American Health Organization/World Health Organization (PAHO/WHO). In 1975, the First International Conference on Conservation of Nonhuman Primates in the New World and their Utilization in Biomedical Research was held, which recommended the development of a Primatology Project.

A Letter of Agreement between the government of Peru and PAHO/WHO was signed in 1975, laying the foundations for cooperation in implementing a biological research project on nonhuman primates—the Peru-

Peruvian Primatology Project

vian Primatology Project (PPP)—with a view to meeting the needs of the biomedical scientific community and at the same time using appropriate techniques to ensure the sustainable management of species in their natural habitat. Within this framework, the Center for the Breeding and Conservation of Primates of Iquitos (CRCP-PPP) was created, which is affiliated with the IVITA Experimental Station.

OBJECTIVES

The objectives of the CRCP-PPP are to generate knowledge and apply techniques for managing neotropical primate species in captivity, contributing to biomedical research and improvements in the quality of life of the population as well as to the sustainable management of Amazon biodiversity. These objectives will be achieved by (1) generating techniques and knowledge for efficient management of primates in captivity, and (2) developing techniques for evaluating and managing primate populations in controlled natural areas (islands).

INFRASTRUCTURE

The CRCP-PPP is located in the Department of Loreto near the city of Iquitos in the IVITA Experimental Station, which has 8 ha of land. It has offices, sheds, a library, a laboratory, storerooms, and electricity and potable water services. It also has a field, a laboratory, and computer materials and equipment, as well as land and water vehicles.

The Padre Island Biological Station comprises an area of 8.3 km^2 and 96 m^2 of buildings with reinforced concrete walls and a corrugated metal roof. The Station has demonstration agroforest plots. The Muyuy Island Biological Station is located 18 km from Iquitos, along the Amazon River. In 1989, a 150 m^2 building was constructed of wood with a sheet metal roof.

ACTIVITIES AND STUDIES

Basically, activities and studies at the CRCP-PPP are for the purposes of breeding and health in captivity and management in controlled natural areas (islands). In this presentation, I will describe the progress and results of activities conducted in recent years.

BREEDING, HEALTH, AND HUSBANDRY IN CAPTIVITY

Most of the research on breeding and health is conducted at the CRCP. In addition to the study on population dynamics of colonies and health

management and monitoring, research has been conducted in the areas of biometrics, breeding, diagnostic criteria, and disease prevalence.

POPULATION DYNAMICS OF *AOTUS* AND *SAIMIRI* COLONIES

After 20 years of operation, we have reached the fifth generation (F5) of *Aotus nancymae* and the third generation (F3) of *A. vociferans*. The breeding activity of the *Aotus* colonies is currently at very satisfactory levels because the colonies consist chiefly of specimens born in captivity, and replacement breeders are selected on the basis of the clinical and breeding history of their parents. Breeding outcomes are also satisfactory in the *Saimiri* colony.

The origin of the breeders in the *A. nancymae* colony has been analyzed. Currently, 75% have been born in the colony, and 25% have been taken from natural areas; the breakdown for the *A. vociferans* colony is 59 and 41%, respectively. Similarly, the origin of the *Saimiri sciureus* colony has been established: 65% have been born in the colonies and 35% have been captured; the composition of the *S. boliviensis* colony is 41 and 59%, respectively.

FEEDING

The colonies consume 45 to 50 g of a wafer-like concentrate and fruit per animal (*Aotus*) or 50 to 60 g per animal (*Saimiri*), per day. The concentrate consists of soybean, wheat, and rice meal, sugar, eggs, vegetable oil, and peanuts obtained locally; premixes of vitamins and specific minerals for nonhuman primates are also included, which are imported from the United States. The nutritional content of the wafer is 24% crude protein, 10% fat, and 4% ash.

HEALTH MONITORING AND CONTROL OF COLONIES

Health monitoring and control are carried out with support from the CRCP Laboratory, especially for clinical analysis of specimens and microbiological monitoring of food and water intake to keep species in captivity in good health. The laboratory also checks the Center's colonies for parasites every 3 months.

Ivermectin and mebendazole are the parasiticides used to fight *Strongyloides* and *Trypanoxiurus*, and metronidazole is used to fight flagellate protozoans. The parasites observed most frequently have been *Strongyloides*, *Hymenolepis*, *Trypanoxiurus*, and flagellate protozoans. In *Saguinus*, *Prosthenorchis* have also been observed.

Necropsies indicate that the leading causes of death in adult *Aotus* have been pneumonia and heart disease, as well as liver degeneration in animals that have been in captivity for more than 10 years. In offspring, the leading causes have been respiratory and enteric infections.

Adult and elderly *Saimiri* present with enteric and respiratory infections (pneumonias). In offspring, the deaths were caused by rejection, cannibalism, and pneumonias in neonates.

GROWTH AND DEVELOPMENT DURING THE FIRST 6 MONTHS OF LIFE

Growth and development parameters for the first 6 months of life were established as basic knowledge for managing *A. nancymae* offspring, which have an average birthweight of 91 g. At the end of the study, the specimens reached an average weight of 494 g (500 g for males and 488 g for females). In Figure 1, the evolution of the weight and length of *A. nancymae* are shown.

In *S. boliviensis*, birthweight does not differ by gender (average weight 99 g). However, when they are 180 days old, males weigh more than females (413 vs. 365 g).

STUDIES TO IMPROVE BREEDING

In the breeding study to evaluate options for lowering the weaning and breeding ages in *A. nancymae*, weaning ages of 5, 6, 7, 8, 9, and 10 months were evaluated. In Figure 2, the changes in weight at 12 months of

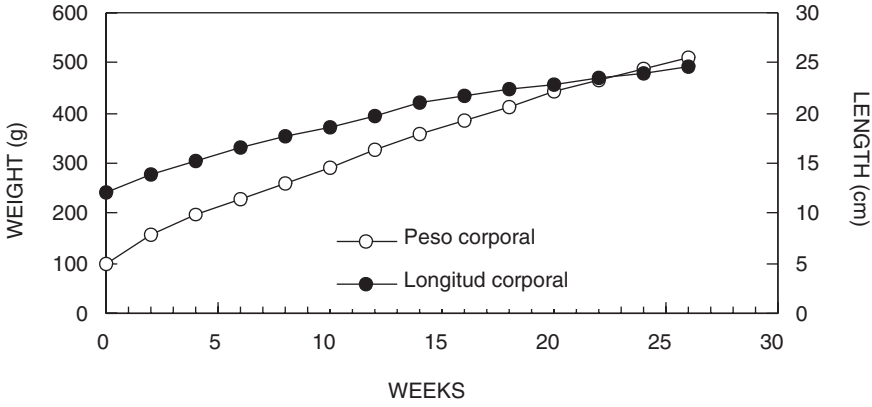

FIGURE 1 Weight and length of *Aotus nancymae*, up to 26 weeks of age: 1998.

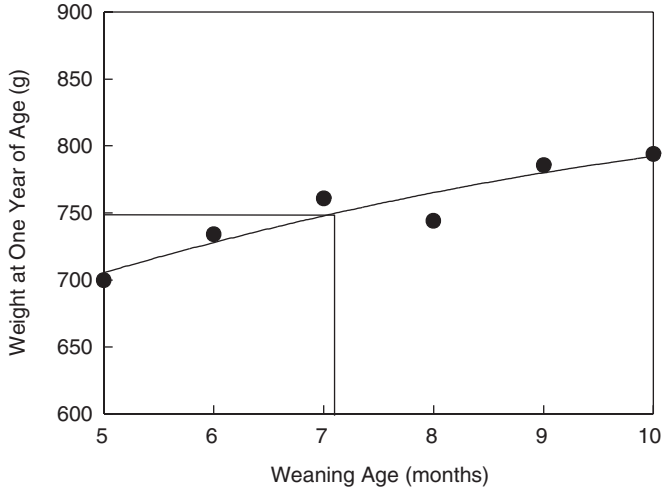

FIGURE 2 Weight at 1 year of age of *Aotus nancymae* in relation to weaning age: 2000.

age in relation to weaning age are shown. We observed that it is possible to obtain specimens weighing approximately 750 g that are appropriate for selection as breeders with a weaning age of 7 months.

A test was conducted with 20 *A. nancymae* females weaned before age 1 and paired at 14 months of age on average, to evaluate the possibility of lowering the breeding age. Of these females, 70% had their first reproductive event after an average interval of 14 months and obtained their first offspring at 28 months of age on average.

STUDIES ON THE PREVALENCE OF ANTIBODIES

The study on prevalence of antibodies against *T. gondii* revealed the following: 13.6% in *A. nancymae*, 12.3% in *S. boliviensis*, and 18.7% in *S. sciureus* in captivity. Recorded prevalence was higher in females and, in relation to age, was higher in juveniles than in adults and subadults. However, there were no clinical manifestations of natural infection.

Various arboviruses cause febrile illnesses in humans in the tropics. To determine the importance of neotropical primates in the transmission of these agents, serum samples were collected from recently captured *A. vociferans*, *A. nancymae*, and *S. boliviensis*. Using the enzyme-linked immunosorbent assay technique, the samples are being analyzed against an arbovirus panel (dengue, yellow fever, mayaro, oropuche, and Venezuelan equine encephalitis).

PHYSIOLOGICAL PROFILES FOR DIAGNOSIS

Under the handling conditions described, it has been possible to establish normal physiological profiles for *A. nancymae* with respect to hematology, blood biochemistry, and creatinine and liver enzyme serum levels. Hematology profiles have also been established for *A. vociferans*, *Saguinus mystax*, and *S. labiatus*; and urea, creatinine, and liver enzyme serum profiles for *S. boliviensis*.

EFFECTIVENESS OF IVERMECTIN 0.1% AND FENBENDAZOLE AGAINST GASTROINTESTINAL PARASITES

Doses of fenbendazole and ivermectin were given to *S. mystax* and *S. fuscicollis* specimens found positive for *Strongyloides* spp., *Trichostrongylus* spp., and *Prostenorchis elegans* from Padre Island and natural areas. The doses effectively controlled *Strongyloides* spp. and *Trichostrongylus* spp. within 72 hours; however, the prevalence of *P. elegans* did not show any variation.

INFLUENCE OF HUMAN COMMUNITIES ON IQUITOS ISLAND ON THE PARASITE LOAD IN CEBIDAE

The sample took persons (n=223) from hamlets on Iquitos and Padre Islands; 97% tested positive for parasitic infections (*Ascaris lumbricoides*, 67%; *Trichuris trichura*, 31%; and *Uncinaria*, 2%). At the same time, in *A. nancymae* (n=132), 91% tested positive for parasitic infections; a *Strongyloides* sp. prevalence of 26% was recorded in simple infections; in mixed infections, the association between flagellate protozoans and *Strongyloides* was 15%. The differences in the parasite profiles suggest that there is no human influence on the parasite load in *A. nancymae*.

CONTROLLED NATURAL AREAS (ISLANDS)

The research on the islands includes the following: (1) evaluating the population dynamics of primates, especially *Saguinus* spp., to estimate the size of the colony it can support and the optimal rate of collection; and (2) establishing alternative land use systems to reduce the pressure of local populations on the primates' habitat.

STUDIES ON POPULATION DYNAMICS

***Saguinus mystax* on Padre Island.** Three consecutive introductions of specimens took place between 1977 and 1980, for a total of 87. Since 1980

the number, size, and structure of family groups have been evaluated, as well as increases in population. In Figure 3, the evolution of the population, which rose consistently except in the collection years, is shown. Even so, the increase resumes after the collection. According to the intrinsic growth rate (without collections), the support capacity was estimated at 428 individuals, which would be reached in 2005; and from that time, growth would begin to stabilize and fall. For the population to maintain its rate of growth, 35 specimens should be removed every 3.5 years.

***Saguinus labiatus* on Muyuy Island.** In December 1989, 31 specimens were introduced on the island, and family groups are evaluated annually to determine population dynamics. In 1996, 54 specimens were surveyed, and the size and structure of three family groups were determined as well as the plant species that make up their diet and the behavior of this species in the presence of human activities. In 1999, of the 20 troops identified, 14 were fully counted and 100 individuals were recorded. The number of pregnant females indicates satisfactory breeding of the species on this island.

Cebidae census on Iquitos Island. In 1998, Cebidae population censuses were conducted on Iquitos Island (*A. nancymae* and *S. boliviensis*) in

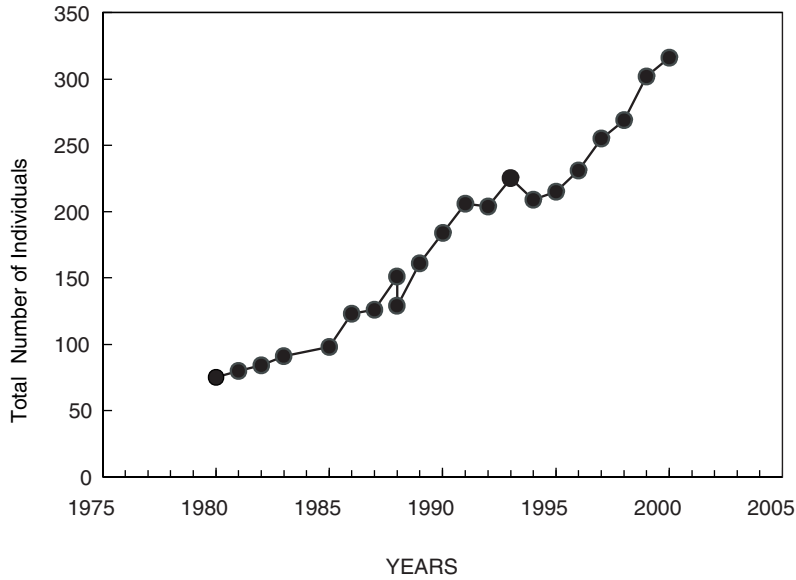

FIGURE 3 Evolution of the *Saguinus mystax* population on Padre Island.

the vicinity of the hamlet of San Pedro de Huashalado. The respective population densities were 35 and 92.3 individuals per km^2.

Breeding behavior and breeding efficiencies of these colonies may be seen in Tables 1 and 2, respectively.

ALTERNATIVE LAND USE SYSTEMS

The populations introduced onto islands must deal with pressure from human groups settled in the environs. One way to reduce the pressure from the inhabitants is by educating communities about the value of primates, in addition to offering options for more intensive land use.

DEMONSTRATION AGROFOREST PLOTS

Since 1993, 7 ha of demonstration agroforest plots of arazá, carambola, camu camu, rice, and yucca on Padre Island have been evaluated. This activity extends to Iquitos Island and the right bank of the Amazon River through the cultivation of seedbeds of fruit and forest species. In 1996, a 0.5-ha demonstration plot was installed on Muyuy Island with the same species.

The plots are maintained and periodically evaluated, and seedlings are transferred to the communities. Currently, the agroforest plots are systematically compared with traditional island production systems.

INSTITUTIONAL IMPACT

The impact of the CRCP-PPP is not limited to its institutional objectives; instead, through academic and social support, it has reached out to

TABLE 1 Breeding Behavior of the *Aotus* and *Saimiri* Colonies: 2001[a]

Species	Initial Population	Breeding Groups	Births	Type of Breeding	Final Population	Breeding Groups
A. nancymae	284	95	87	Pairs	338	106
A. vociferans	165	53	44	Pairs	203	61
S. boliviensis	154	15:76	41	Harem	164	21:83
S. sciureus	39	4:21	5	Harem	38	6:23
TOTAL	642	743				

[a]Breeding efficiency has been consolidated (number of offspring born/number of breeding females × 100) (Table 2) through the maintenance of breeding records, gradual renewal of the breeding group, adequate diet, the rehabilitation of infrastructure, and disease surveillance and control.

TABLE 2 Breeding Efficiency in Captivity: 2001

Species	Breeding Groups	Reproductive Event[a]			Breeding Efficiency
		LB	SB	MC	
Aotus nancymae	95	87	2	6	91%
Aotus vociferans	53	44	4	5	83%
Saimiri boliviensis	17:76	41	5	54	
Saimiri sciureus	6:21	5	24		

[a]LB, live births; SB, stillbirths; MC, miscarriages.

the community. It regularly hosts academic events such as courses and training for students from the UNMSM Schools of Veterinary Medicine and Biology, as well as from other national and international universities and local technical institutes.

One effective social outreach focus is the dissemination of information on alternative island land use systems when talks are given on ecodevelopment, agroforestry, wildlife management, human health, and the use of primates in research, as well as technical assistance in community farming areas. During these activities, the creation of family agroforest plots is supported. In addition, since 1996, 10,080 grafts of cedar, huito, arazá, carambola, and camu have been distributed to 60 beneficiary families in nine communities on Padre and Iquitos Islands.

The publication of research findings has been abundant. There are articles in science journals, abstracts at scientific congresses on this specialty, and institutional reports. As part of this effort, the publication of *La Primatología en el Perú* (Primatology in Peru) Volumes I and II is noteworthy.

Finally, there have been periodic transfers of neotropical primates of biomedical interest to scientific institutions through PAHO/WHO. The numbers are estimated at 250 specimens per year in the past decade. Biological samples were also sent for specific studies.

OUTLOOK

The research and outreach activities during the period make the CRCP-PPP a sort of precursor in science and biomedical culture in the area of influence. Its performance has appreciated the value of nonhuman primates for medical research and has raised awareness in some interest

groups about the value of the country's biodiversity. The progress and results must be consolidated through the following initiatives:

- Validation of techniques for captive and semicaptive (island) breeding of species of scientific and cultural interest and for recognition and treatment of spontaneous diseases.
- Contributions to the knowledge of captive state physiology, nutrition, breeding, pathology, and behavior of native species.
- Generation of semicaptive state (islands) population management techniques, which include agroforestry and enrichment of the forest for its rational use. This experiment, with the participation of local communities, is likely to extend to other species of Amazon fauna and flora, including research on and management of medicinal plants. The CRCP-PPP is a pioneering experiment in the management of nonhuman primates of biomedical interest.
- Sustainable use of nonhuman primate populations of biomedical and cultural interest through the development of management techniques, together with studies on biodiversity and forest productivity. Our experience indicates that the periodic collection or capture of species, such as *A. nancymae*, *A. vociferans*, *Saguinus mystax*, *S. fuscicollis*, *S. boliviensis*, and *S. sciureus*, is feasible. Collection expeditions should also be used to study the biology of the species in natural areas and their importance in maintaining biodiversity and forest productivity.
- Inventory and identification of primate species in Peru, as well as their geographic distribution and population dynamics. This strategy would protect endangered species and would make it possible to determine their geographic distribution and population dynamics (density and group composition). Authorities must step up environmental education efforts and support for control officials to achieve effective protection and conservation.

Session 2: Panel Discussion

Participants:
John G. Vandenbergh—Session Chair, North Carolina State University, USA
Gerhard Hunsmann—Deutsches Primatenzentrum, Germany
Boris A. Lapin—Institute of Medical Primatology, Russia
Jerry Robinson—NIH/NCRR, USA
Greg Beattie—Sierra Biomedical, USA
Enrique Montoya—Peruvian Primatology Project, Peru

QUESTIONS AND ANSWERS

DR. VANDENBERGH (John G. Vandenbergh, North Carolina State University): One of the concerns that occurred to me as I was sitting here is that we are talking about a resource, the nonhuman primate, that is becoming more and more limited and more and more difficult to work with in many ways. Dr. Lapin and some of the other speakers referred to the idea that we need to have highly justified pieces of research on this. We have heard our opening speaker today, Dr. Hearn, talk about strategic research versus basic research.

What concerns me, as the nonhuman primate becomes a more limited resource, is whether we will focus almost entirely on the strategic and the applied and will dry up that well of basic information that we need. I wonder whether the panel would be interested in responding to that

concern: whether we will have continued opportunities for basic research as well as important drug testing and other very applied things that we need.

DR. ROBINSON (Jerry Robinson, National Center for Research Resources): You had addressed the important issue with respect to the primate centers, and that part of the original charge of the primate centers was to do conservation and field studies. However, with the cost of biomedical research skyrocketing as it is, the ability is very limited to provide resources for those people to do those important studies in the wild as well as maintain the animals in captivity.

DR. VANDENBERGH: And not just the wild as we all understand. I am also concerned about the laboratory aspect.

DR. HUNSMANN (Gerhard Hunsmann, Deutsches Primatenzentrum): We at the German Primate Center have a substantial amount of our overall funds being used for field studies. That amount is increasing, actually. The new philosophy now is that biomedical research must carry its own weight meaning that money for it must come from outside—either grants or contracts. We have studies in the field (e.g., a field station in Madagascar and the University in Iquitos), where studies are supported by the core grant of the primate center. It is a little different from what we hear is going on in the United States.

DR. ROBINSON: I would like to add that NCRR, through their small instrumentation grants and so forth, serves the primate facilities that engage in imaging small animals. A great deal of physiological basic understanding is being derived from such studies.

PARTICIPANT: It is my perception that at least with regard to rhesus, baboons, and chimpanzees, there has been a sort of waxing and waning of the needs and the demands for those species over time. Whenever the demand has built up, there has been a response from NIH to produce more animals and make them available for research. When there have been enough animals, or demand has decreased because scientific needs change every 5, 10, or 15 years, there has been the sense that these animals have been in excess and therefore should be reduced to keep the costs down. If that perception is correct, it is my sense that perhaps it would be more economical to maintain a surplus of these critical species as insurance so that when demand picks up again (and it always seems to because it always has as long as I have been working with nonhuman primates), it is actually possible to meet that demand quickly. That approach might actually be less expense than allowing the numbers to decrease to exactly what the demand is during a period of low demand.

So, I have two questions. First, is that your perception also or do you see the situation somewhat differently? Second, if your perception is the same, can you envision any way of changing the priorities not to have this

waxing and waning supply that never seems to be in synchrony with demand?

DR. ROBINSON: I totally agree with your perception, which I tried to express in the table I showed with the primate center breeding colonies. Yes, it does appear to come and go. In primate centers in the past (e.g., when I was at Wisconsin), it seemed as if we maintained animals for long periods of time. However, with regard to the long-term strategy, perhaps this latest crunch is a wakeup call for the government to maintain certain levels of nonhuman primates so that they are available. I mean, bioterrorism issue is a key example because they suddenly need to retest all of these vaccines and so forth, and the animals are not available.

DR. ERVIN (Frank Ervin, McGill University): Having sat on NIH review committees for well over 25 years, I would like to question Dr. Robinson's statement that of the 13,000 NHP used by NIH investigators every year, 65% of which are rhesus, that a significant number of rhesus were required for experiments. In fact, most of them did not need a rhesus macaque. They just thought they would like to have a rhesus. For some of them, that was the only macaque they could spell. For some of them, it was the one that was available in the local colony. For some of them, their major competitor in the field had used a rhesus, but there was no biological justification for using a rhesus. If you would like to increase your rhesus availability, Jean Baulu in Barbados and Frank Ervin in St. Kitts can cover half of your use of wasted rhesus and use them for something else.

DR. ROBINSON: I totally agree, and I think one of the things NCRR is trying to do is to make investigators more aware of other nonhuman primate species to make them consider the most appropriate biological model.

DR. ERVIN: It seems to me that concept is terribly important. Not only do they have to use a monkey, but do they have to use a particular kind of monkey? Some people only need a monkey.

DR. ROBINSON: I would also like to say that part of an experimental design must go through their IACUC. That is the very first thing IACUC members look at—whether a monkey is really required to do an experiment. Then, given the availability of other potential nonhuman primates at that particular center, if they can direct an investigator to use those other nonhuman primates species, they do so.

DR. BELOTTO (Albino Belotto, Pan American Health Organization): First, I would like to add that at the Peruvian Primatology Project, we have worked together with the Pan American Health Organization and the Bolivian government for the last 25 years. We consider it a very successful project. In the beginning, we were very fortunate to have strategic support from NIH.

We have had the transfer of animals that Dr. Montoya mentioned, through our very successful association with NIH and Dr. Taylor. We, of course, would like to continue this work. We follow the legal process of the Peruvian government. Now that I hear about NIH providing support not only for research itself but also for breeding and primate development, I would like to know how to obtain financial support from Peru as well as from NIH.

DR. ROBINSON: Let me understand the question correctly. You are asking how a foreign country applies for NIH funding. Unfortunately, NCRR, which is the only institute I can speak for in this regard, does not directly support primate facilities through a grant mechanism like that. However, if there is an affiliation with a US institution (e.g., Indonesia with the Washington Primate Center), we provide support through the Washington Primate Center grant that supports that breeding facility in Indonesia. That is one example.

PARTICIPANT: I have one question and one comment. Dr. Beattie, I saw the data you mentioned presented at the Association of Primate Veterinarians in the context of lymphocyte subpopulations between different geographic origin cynomolgus. I would like to know whether any of that information has been published because I think it is critical in understanding the variability of populations and giving us better insight, not regarding whether one population is preferable over another as much as to characterize the populations we are working with and understanding the relevance to particular experimental designs. Could you comment on that?

DR. BEATTIE (Greg Beattie, Sierra Biomedical): The data have been presented at various meetings including the Society of Toxicological Pathologists as well as the Society of Toxicology meeting just this past year. They have not been formally published as a journal article yet; however, we anticipate publishing it soon. People such as yourself, and even people internally, ask regularly. We are using the data currently to select the species in a geographic region for which we will continue a program.

We are seeing that clients who have started a program have different responses based on their studies up to a certain point. We may not know exactly where the animals came from, but we end up with a 3-month study with different responses that we cannot interpret.

PARTICIPANT (CONTINUED): The University of California at Davis was originally the national center for primate biology, and now it has gone "full circle" and is the national/regional center for primates. I was struck by the original opening comments about availability of opportunities for research on the basic biology of species and that there will be more focus on drug development, which particularly affects the primate centers program. When they revised the base grant format, they elimi-

nated the RO1-type research, but they introduced the mechanism of resource research projects. We took advantage of that revision at Davis, looking at basic biological aspects. One project we are considering is biobehavioral characterization of very young animals. I think when you go back to that basic concept, looking at the biology of these species and understanding more about population variability, you go back to NCRR and the primate center's program, which is a real core value that has again been emphasized in the primate center's program.

DR. STEWART (V. Ann Stewart, Walter Reed Army Medical Center): I would like to point out that one of the statistics that was relatively hidden in that USDA figure is that there are two kinds of nonhuman primate protocol. There are protocols that use primates, and there are protocols that use primates up. Certainly, there are good scientific justifications for continuing with the species once you have started with it in a program. One of the reasons for the current rhesus shortage is the fact that the share of the research became a one-way trip for a lot of these animals. Clearly many of the toxicology studies are also a one-way trip for a lot of these animals. So I am wondering whether it is perhaps time to develop some sort of policy about categorizing or prioritizing research needs based on whether they are protocols that use rhesus or use rhesus up—whether we can try to create some sort of program for getting these animals into a couple of shoot and bleed types of studies and then have them go to SHF or toxicology studies.

PARTICIPANT: I would like to comment that at the primate centers, this practice is in place. Although I do not like using the term, animals are "recycled," if you will. In the chimpanzee program, for example, the National Institute of Allergy and Infectious Diseases program, they begin with young chimpanzees and study respiratory virus. Then the study progresses to other types of experimentation, and then it may end up with, for example, hepatitis. I would like to return to what Dr. Hearn said this morning about the development of the technique to recover embryos from rhesus macaque, which enables those animals to continue to be reproductively active. Although there are old methods of doing multiple surgeries on an animal to recover embryos like that, there are limitations on the things that can be done. So that is another example.

HANS-ERIK CARLSSON (Hans-Erik Carlsson, Uppsala University): I would like to hear the panel's comment on the number of animals used. We are currently conducting a survey and looking at all of the published studies on primates used during 2001. We have found that approximately 3500 studies have been done. The number of animals used in the United States appears to be around 17,000, which agrees with Dr. Robinson's presentation, except for the USDA's figures, which totaled about 60,000 animals in the United States, I believe. Do you have a comment on that?

DR. BEATTIE: The USDA's number was about 56,000, as I remember, for 2000, and yours was much less than that. However, I was dealing only with NIH grantees. The USDA covers the pharmaceutical companies. They are required to report how many nonhuman primates they use as well. Whereas the survey I was dealing with was just slightly over 1000 NIH grantees, actually only 641 of them reported back.

HANS-ERIK CARLSSON: So that means that a large number of animals used in studies are not published?

DR. ROBINSON: Not necessarily. They are reported to the USDA, but that does not mean that they are reported to the NIH, because the NIH does not support all of those studies. In other words, of the large number of studies done, only a fraction is reported through the NIH mechanism because the people hold grants.

DR. BEATTIE: It is my understanding that the pharmaceutical companies are required to report the number of animals they use through the USDA, but the USDA does not require a breakdown other than to identify them as nonhuman primates.

DR. BAUDOIN (Mario Baudoin, Ministry of Sustainable Development and Planning): Before I announce the appointment of Dr. Ervin as my marketing director (laughter), I have a question for Dr. Hunsmann from the German Primate Center. You mentioned the total number of primates imported in the European Union, and you should add to that the 800 green monkeys we have sent each year to those countries for at least the last 10 years. The important thing is that you may have considered only the macaques. Alternatively, if you did ask for other species, I understand very well why you did not get the answer because most private companies, pharmaceutical companies, are very reluctant to report how many monkeys they use when they are producing vaccines. This reluctance is simply to protect themselves or at least keep proprietary information or whatever.

I also would like to ask why in Iquitos the breeding efficiency is only 5% with saimiri, whereas there is much greater success with marmosets.

DR. ROBINSON: There were only five animals—a very small number.

Session 3

Nutrient Requirements of Nonhuman Primates

Nutrient Requirements of Nonhuman Primates[1]

Committee on Animal Nutrition, Board on Agriculture and Natural Resources, NRC

OVERVIEW

Nutrient requirements of monkeys were first considered by the National Research Council's Committee on Animal Nutrition in a section of *Nutrient Requirements of Laboratory Animals* (National Research Council, 1972). The information was updated and expanded in *Nutrient Requirements of Nonhuman Primates* (National Research Council, 1978). The present publication is a second revised edition of the 1978 report that constitutes a further updating and expansion of the topic (National Research Council, 2003).

This report is distinctive among most other publications in the Committee on Animal Nutrition series of reports on animal nutrient requirements. Many of the reports in this series deal with a particular species of domestic animal for which there is a significant amount of peer-reviewed research and an abundance of studies that examine specific nutrient requirements for various life stages. This revision is unlike those other reports for several reasons. First, it attempts to address the needs of over 250 species. Second, there are few data on which to draw conclusions and make recommendations for most species. Third, the animals addressed here are not domestic animals raised and bred for maximum efficiency in

[1]Reprinted from National Research Council (2003).

growth and production, but rather they encompass research animals, educational animals, and rare, endangered, and threatened animals that are maintained in various institutions for conservation purposes. Given the nature and importance of the animals that are the topic of this report and recognizing that the users of this report will span a wide range of professional expertise and practical knowledge of nutrition, the Committee used extreme care in evaluating and summarizing the available information. We chose not to go beyond what the data allow and we have grounded our recommendations firmly in scientific fact. To deviate from this approach, to venture beyond the scientific evidence, or to attempt to provide equations and estimates that cannot be validated—as they are validated in domestic food-producing animals—could potentially do more harm than good to the approximately half million primates currently maintained in biomedical and conservation institutions throughout the world.

Definition of the nutrient requirements of a single primate species at all life stages is difficult because little research specifically aimed at determination of nutrient requirements has been conducted. Definition of the nutrient requirements of each of some 250 primate species is virtually impossible with our current knowledge. Energy requirements of fewer than 20 species have been studied, and protein, mineral, and vitamin requirements of fewer than 10. Although there may be much dissimilarity among primate species in behavior and in the presence of fermentation compartments within the gastrointestinal system, similarities in the other aspects of physiology that influence nutrient requirements tend to be greater than the differences. Some extrapolation from one species to another is possible; this allows the formulation of diets that will usually meet requirements for adult maintenance, reproduction, and growth, even though specific quantitative needs have not been experimentally established. Although much more information is needed in those instances where specialized features of the gastrointestinal tract dictate a comparably specialized diet, research findings are beginning to fill the knowledge gap.

With few exceptions, captive species can be sustained in good health for periods equal to or greater than their life spans in the wild. That does not mean that all institutions housing primates are equally successful, but such an outcome is probable if rational and research-based dietary practices are consistently followed. This document is meant to help those who are struggling with this challenge.

When defining nutrient requirements, it is common to search for minimal dietary concentrations that will support maximal responses in important end points, such as growth rate of the young. It would be ideal if the same nutrient concentration produced a maximal response in all impor-

tant endpoints, but that is seldom the case. For example, vitamin E has little effect on growth rate but is exceedingly important in protecting cellular membranes against the peroxidative damage associated with the stress of capture and handling. Furthermore, the degree of protection appears to be positively related to the dose until tissues are fully saturated; to complicate the matter, tissues of some organs become fully saturated with vitamin E before tissues of others. Thus, as satisfying as it would be to have a single minimal dietary concentration that met the requirements of the whole animal, minimal required concentrations vary with the sensitivity of the endpoint selected. Because nutrient-requirement research in primates is so sparse, we have seldom had the option of identifying a need for more than one end-point. When such information was available, we tried to relate the minimal requirement to it.

Chapter 1 is a new feature of this revision that was not provided in the previous edition. This chapter is provided to give the reader an understanding of variations in feeding ecology and digestive strategies among primates, which is critical knowledge needed to make informed decisions on feeding primates. The discussion is concerned with foraging strategies in natural ecosystems, species differences in gastrointestinal morphology and physiology, and the significance of these factors in development of appropriate systems of dietary husbandry for captive primates. Because the usefulness of data gathered in field studies of feeding ecology varies with the method used, we discuss the strengths and weaknesses of the methods. Relevant field-study data are tabulated by species, and we illustrate the various gastrointestinal types found among nonhuman primates.

Chapter 2 is a detailed review of energy terms, methods used to determine energy requirements, and energy requirements of nonhuman primates for adult maintenance, growth of young, and pregnancy and lactation. Tables include data on body weight, measured energy expenditures, and estimates of daily metabolizable-energy requirements as multiples of basal metabolic rate.

Chapter 3 discusses first the classification of carbohydrates, their characteristics, digestion, metabolism, and analysis and then discusses analytic systems for fiber, the role of dietary fiber in primate gastrointestinal health, and potentially beneficial dietary fiber concentrations.

Chapter 4 covers proteins, protein sources, and methods of assessing protein quality and requirements. Information on protein-calorie malnutrition and on protein deficiencies and excesses is included. Although quantitative requirements of nonhuman primates for specific amino acids could not be defined, evidence of the essentiality of methionine, lysine, phenylalanine, tryptophan, and taurine is presented. Protein requirements, based on high-quality reference proteins and various criteria, are given in tabular form.

Chapter 5 addresses fats and fatty acids, including classification, nomenclature, digestion, absorption, and metabolism. It describes essential fatty acids and presents estimated requirements for n-3 and n-6 fatty acids. Fatty acid composition of primate milks, potentially harmful fatty acids, cholesterol metabolism, and use of nonhuman primates as models for study of cardiovascular disease are discussed.

Perhaps the most greatly expanded chapter in this revision is Chapter 6, which is a review of mineral nutrition and metabolism, including functions and signs of mineral deficiencies and excesses. In the first edition of this report, which was published in 1978, there was no discussion of sulfur, copper, cobalt, or molybdenum needs of nonhuman primates. In Chapter 6 of this second edition, we are able to provide the first recommendations on mineral requirements for copper and selenium based on a comprehensive review of the scientific literature. Similarly, Chapter 6 provides the first review and discussion of sulfur and cobalt in primate nutrition by the National Research Council Committee on Animal Nutrition. Mineral requirements of several primate species at various ages are given.

Chapter 7 is a discussion of fat- and water-soluble vitamins, including form, function, metabolism, and signs of deficiency and toxicity. Estimates of quantitative requirements of nonhuman primates are provided.

Chapter 8 deals with water as a component of the primate body and with the influence of activity and various environmental factors on the proportion of body water. Water sources, water quality, water turnover, water requirements, and important considerations in providing water for nonhuman primates are discussed.

Chapter 9 presents information on a number of pathophysiologic and life-stage considerations that are relevant to nonhuman-primate nutrition. It includes values of body mass (weight) and body composition, studies of the nutritional needs of neonates, effects of aging on nutritional needs, and relationships of nutrition to aging, obesity, and diabetes. Special considerations for hand-rearing of orphaned or abandoned young animals are covered and recommendations for simulating the composition of milk produced by the mother in normal lactation and the mother's normal nursing schedule are provided as well as introducing solid food into the diet as the young progress toward weaning.

Chapter 10 discusses primate-diet formulation, effects of feed processing on nutrient loss, factors that influence food intake, and some general suggestions for dietary husbandry. Plants that have been safely used as browse offerings in captivity are listed.

Providing much more detailed and focused recommendations than the general recommendations provided in the previous edition, Chapter

11 tabulates estimated nutrient requirements of model nonhuman primates in six categories (suborder Strepsirrhini; families Hominidae and Pongidae, Cercopithecidae, Cebidae, and Callitrichidae; and subfamily Colobinae). These requirements were estimated on the basis of a thorough review of the world's scientific literature, input from numerous scientific sources, and the Committee's best judgment. The requirements apply most satisfactorily to purified diets with high nutrient bioavailability and without substantial adverse interactions among nutrients. The estimates represent minimal requirements without safety allowances.

Also provided in this chapter is a table (Table 11-2) of dietary nutrient concentrations proposed as a guide for formulation of diets containing natural ingredients and intended for post-weaning primates. These have been expressed per unit of dietary dry matter, assuming an energy density of 4 kcal ME·DM_g^{-1}. It should be noted that these nutrient concentrations are intended only as guides, have not been directly tested as a group with any primate, and may not be appropriate for all species or all post-weaning physiologic stages.

Chapter 12 provides tables of the compositions of feeds commonly used in nonhuman-primate diets.

Chapter 13 is a new area of discussion that was not included in the previous edition. This chapter discusses food as a component of environmental enhancement, an application arising from concern for the psychologic well-being of nonhuman primates in captivity. Various food choices and means of presentation are suggested.

The Appendix contains a scheme of taxonomic relationships within the Primate Order, including scientific and common names, plus tables of weight equivalents and weight-unit conversion factors.

The Committee has concluded that appropriately formulated nutritionally complete diets best serve the health and welfare needs of most captive primates. These diets are available in various forms including dry extruded, canned, and gelled. Potential impacts on oral health are among the many factors that must be considered when selecting the form of a diet to be fed.

If fed as size-appropriate, ground, mixed, dry extrusions, oral health will not be compromised. It initially might be necessary to entice some animals to accept dry extrusions by softening them with water, mashed fruit, fruit juices, or nectars. Other foods can be used for behavioral enrichment, but care must be exercised to ensure that their composition and amounts consumed do not distort nutrient concentrations and ratios in total dietary dry matter beyond required minimums and maximums. In general, alternative foods that are high in moisture are least likely to have such effects.

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Session 4

Genetics

Nonhuman Primates in Genetic Research on Common Diseases

John L. VandeBerg, PhD,[*,†] and Sarah Williams-Blangero, PhD*[†]

The leading causes of death in the United States are heart disease and cancer, with diabetes ranking sixth (Minino and Smith 2001). In addition to the health burden of these multifactorial diseases, progressive chronic conditions such as osteoporosis are associated with significant costs for health care and quality of life (Siris and others 2001; Tosteson and others 2001). From a global perspective, parasitic diseases such as schistosomiasis and Chagas' disease (American trypanosomiasis) present tremendous health burdens in developing countries (Chan 1997; Murray and Lopez 1997).

Genetic approaches to these leading causes of morbidity and mortality seek to characterize the genetic components that influence susceptibility to disease processes. Statistical and molecular genetic techniques are used to quantify genetic influences on disease-associated traits and, ultimately, to identify the specific loci determining patterns of variation. Knowledge of the genes responsible for susceptibility can be used to target treatments or recommend lifestyle changes (e.g., dietary restrictions) to the individuals most likely to develop disease. This information can

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also facilitate drug discovery through identification of biological mechanisms to serve as novel targets in pharmacological development (Dykes 1996; Gelbert and Gregg 1997).

The dramatic progress in genetics that has occurred in the last decade has revolutionized the study of genetic susceptibility to complex diseases. The development of the human gene map, sequencing of the human genome, technological improvements that allow rapid large-scale genotyping of population samples, and advances in statistical genetic methods have created unprecedented opportunity for genetic research on common complex diseases.

For disease processes that occur at a frequency of 10% or greater, an analytical design utilizing extended pedigrees drawn at random from the population (i.e., not selected with respect to disease characteristics) is optimal (Almasy and Blangero 2000). The statistical power of this approach is a function of the size and complexity of the pedigree (Blangero and others 2000; Dyer and others 2001). Extended pedigrees that are not selected on the basis of disease phenotype are useful for analysis of any normal or disease-related trait that is common in the population. Thus, once genotype data are generated for a study of a given common disease, the pedigree becomes an invaluable resource for studies of other traits.

Nonhuman primate colonies frequently have complex pedigree structures, making them well suited to genetic analyses of common diseases. Nonhuman primates serve as excellent models for human disease studies because of their phylogenetic proximity to humans, the large degree of conservation of gene maps between human and nonhuman primates, the genetic and physiological similarities between humans and nonhuman primates, and the natural occurrence of many of the complex diseases that represent the greatest health burdens to the human population (VandeBerg and Williams-Blangero 1996, 1997).

Many complex diseases, including heart disease, diabetes, hypertension, osteoporosis, schistosomiasis, and Chagas' disease, occur naturally in at-risk nonhuman primates. However, genetic studies in nonhuman primates should be directed toward those diseases for which nonhuman primates offer scientific advantages, rather than simply toward those for which nonhuman primates are suitable animal models.

For example, the baboon is an excellent animal model for studies of pathology and immunology in schistosomiasis (Nyindo and Farah 1999). However, it is not an ideal model for studying the genetic determinants of susceptibility to infection with *Schistosoma mansoni*. First, complex extended human pedigrees are available in areas that experience high rates of disease prevalence (Bethony and others 2001; Marquet and others 1996), whereas it would be impractical logistically and financially to subject the

number of pedigreed nonhuman primates needed for a genetic epidemiological study to a challenge with *S. mansoni*.

In contrast, the baboon is an excellent model for genetic studies of susceptibility to another parasitic infection, American trypanosomiasis or *T. cruzi* infection. Chagas' disease is the leading cause of heart disease in Latin America, and it can result in a short-term acute illness or a long-term chronic condition characterized by progressive cardiomyopathy or megaesophagus and megacolon. While large extended human pedigrees with high rates of infection are available for genetic study (Williams-Blangero and others 1997), parallel genetic studies in baboons can shed light on the genetic determinants of pathology and progression of other correlates of infection that are impractical to quantify over time in a longitudinal human study (Williams and others 2000). For example, regular tissue biopsies and radiographic assessments are possible with nonhuman primates to a degree not feasible for human populations living in the remote rural areas where the disease is prevalent.

Nonhuman primates are ideal models for genetic studies of complex disease processes that have significant environmental components. For example, the level of dietary control possible with pedigreed nonhuman primates allows explicit assessment of the interactions between genetic effects and dietary effects in determining physiological correlates of heart disease.

Genetic epidemiological studies of atherosclerosis and its correlates in the baboon model provided the first documentation of a genotype by diet interaction effect for serum cholesterol variation in a primate (MacCluer and others 1988). This was the first explicit evidence for a genetic basis to response to dietary saturated fats and cholesterol. A Program Project from the National Heart, Lung, and Blood Institute (P01 HL28972) has supported research on the genetics of cardiovascular disease risk factors in the pedigreed baboon colony at the Southwest Foundation for Biomedical Research for the last 20 years. The initial documentation of a genotype by diet interaction effect on cholesterol variation by MacCluer and colleagues (1988) has subsequently been refined, and numerous aspects of lipoprotein variation in response to diet have been investigated (e.g., Mahaney and others 1999a; Rainwater and others 1998, 1999). With the completion of a baboon framework gene map (Rogers and others 2000) and the genotyping of all animals in the pedigreed colony for approximately 325 markers spaced evenly across the genome, linkage analyses are being pursued to localize and ultimately to identify the individual genes involved (Cox and others 2002). The discovery of genetic effects and dietary interaction effects on cardiovascular disease risk factors was made possible by the ability to experimentally manipulate the diet in the

baboon model, a technique not possible in large-scale studies of human populations.

Osteoporosis is a major health problem in the United States. One of the primary risk factors for development of osteoporosis is low bone mineral density. Genetic studies of this disease in human populations are hampered by the need to assess bone mineral density in the large numbers of related individuals required for genetic epidemiological analysis and the immeasurable variability in lifetime diet and exercise patterns. The same baboon population studied for the cardiovascular disease studies was assessed for bone mineral density, taking advantage of the pedigree and genotypic data generated for the population. Measures of bone mass and bone mineral density traits exhibit moderate to high heritability in baboons, with between 40 and 67% of the variation attributable to genetic factors (Kammerer and others 1995). A preliminary genome screen for genes influencing bone mineral density traits and other correlates of osteoporosis has localized genes with significant genetic effects on chromosomes 6, 11, and 12 (Mahaney and others 1997, 1999b). These results suggest that the baboon model will be informative for fully characterizing the genetic components of susceptibility to osteoporosis. As is the case with heart disease, knowledge of the genes involved in determining susceptibility may eventually allow targeting of diet and exercise programs to those likely to develop disease and may ultimately lead to new pharmacological interventions.

The examples above illustrate the great utility of nonhuman primate models for characterizing the genetic components of complex diseases that are major health burdens throughout the developed and developing world. Linkage analyses localizing genes with significant effects on trait variability have been conducted for a broad range of disease traits in the single pedigreed baboon population, indicating the tremendous value of a pedigreed and genotyped colony for biomedical research. Already, genes influencing risk factors for cardiovascular disease, Chagas' disease, osteoporosis, hypertension (Kammerer and others 2001), and hormone levels (Martin and others 2001a,b) have already been localized in this population. Ongoing studies are assessing the genetic components of temperament traits (e.g., Kaplan and others 2001a,b) and correlates of psychiatric disease (Jeffrey Rogers, unpublished data) in the baboon model. Future research will focus on the detailed characterization and ultimate identification of quantitative trait loci.

Clearly nonhuman primate colonies have tremendous potential for use in the identification of genes that influence common diseases. However, the utility of nonhuman primate colonies for genetic research relies on several key sets of data. First and foremost, detailed pedigree records must be available. If single male breeding groups are used, pedigree data

alone may be sufficient for reconstruction of pedigrees to be used in genetic epidemiological analysis. If multimale breeding groups are used, genetic marker data will be critical for resolving paternity and maternity errors.

The identification of specific genes with significant effects on disease traits requires linkage analysis of genetic marker data in conjunction with disease trait data and information about the distribution of genes across chromosomes. Although a gene map exists for baboons, none is available for any other nonhuman primate species. The development of gene maps for nonhuman primate species commonly used in biomedical research will be another critical step in developing genetic research with nonhuman primates.

Just as the human genome sequence has been extremely informative for investigators trying to move from gene localization to gene identification in human studies, genome sequence data will be extremely valuable for genetic research with nonhuman primates. The priority for sequencing should be placed on the species most commonly used in biomedical research and, particularly in genetic epidemiological research, the baboon and the rhesus macaque.

There is tremendous potential for future genetic research on nonhuman primates. However, progress in genetic research with nonhuman primates will require a significant investment in the development of pedigreed colonies of nonhuman primates, genotyping and gene mapping efforts in species to be used for genetic research, and the development of sequence information for genetically well-characterized species. Genetic management and improvements in genetic resources will be critical if the benefits of the genome revolution are to be fully realized in research with nonhuman primates.

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Genetic Considerations in the Management of Captive Nonhuman Primates

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INTRODUCTION

Genetic management is an important component of the management of nonhuman primate colonies regardless of whether the animals will be used for genetic or nongenetic research (VandeBerg 1995; Williams-Blangero 1993). Genetic management techniques can be used to maintain the long-term viability of nonhuman primate colonies for continued production of healthy breeders. In addition, genetic management approaches can be used to generate well-characterized research subjects for genetic studies and to allow selection of optimal groups of unrelated animals for use in nongenetic experimental protocols.

Genetic considerations are important at multiple levels for the management of captive nonhuman primate populations. Genetic variability between subspecies, between geographic groups, and within populations has important implications for both management and research (Williams-Blangero and others 2002). The genetic characteristics of the breeding population significantly influence the productivity and stability of a captive nonhuman primate colony.

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GENETIC VARIATIONS BETWEEN SUBSPECIES

Subspecies of nonhuman primates are not always easy to distinguish on the basis of physical characteristics. However, significant genetic differences between subspecies may affect the reproduction rates in mixed-subspecies populations and the experimental utility of hybrid animals (Kohn and others 2001; Moore and others 1990; VandeBerg and others 1990b; Williams-Blangero and others 1990). Genetic variation between subspecies as assessed by genetic markers is expected to be reflected in differences for biomedically relevant traits between subspecies.

For example, significant genetic differences exist among the baboon (*Papio hamadryas s.l.*) subspecies maintained at the Southwest Foundation for Biomedical Research (Williams-Blangero and others 1990). The genetic distances between the subspecies are mirrored by differences in phenotypic variability for lipoprotein traits (Williams-Blangero and Rainwater 1991; Williams-Blangero and others 1990). The baboon population at the Southwest Foundation is being used in ongoing genome scans for genes influencing risk factors associated with cardiovascular disease and osteoporosis (VandeBerg and Williams-Blangero 2002). The animals are predominantly olive baboons (*P.h. anubis*), but a significant amount of admixture with yellow baboons (*P.h. cynocephalus*) has occurred in the past. Subspecies admixture, as measured by percentage of genes derived from the *P.h. cynocephalus* subspecies, has been identified as an important covariate in genetic analyses of both lipoprotein levels and bone mineral density (Mahaney and others 1995, 1999a).

Ideally, nonhuman primate colonies should be composed of a single subspecies. However, if admixture has occurred in the past, breeding histories can be used to estimate individual admixture in terms of percentage of genes derived from the less predominant species. This measure can then be used as a covariate in genetic analyses of data from hybrid animals, and as a means for identifying hybrid individuals to be eliminated from the breeding population.

GENETIC VARIATIONS BETWEEN POPULATIONS WITH DIFFERENT GEOGRAPHIC ORIGINS

Significant genetic differences that have biomedical relevance may exist between populations of the same species derived from geographically distinct regions. Therefore, it is important to consider between-population genetic differences even when subspecies are not formally recognized. The differences between rhesus macaques (*Macaca mulatta*) derived from India and those of Chinese origin clearly demonstrate the relevance

of considering geographic origin in structuring breeding colonies of nonhuman primates for biomedical research.

The ban on importation of macaques from India has resulted in the evaluation of rhesus monkeys of Chinese origin for the many biomedical research programs that rely on macaques, including those related to AIDS research. Significant differences between Indian and Chinese macaques have been documented for morphological, behavioral, physiological, genetic, and immunological characteristics (Champoux and others 1997; Clarke and O'Neil 1999; Joag and others 1994; Marthas and others 2001; Viray and others 2001), suggesting that these two types of rhesus monkeys should not be interbred in captive colonies, except for special research purposes. Recent work by Marthas (Marthas and others 2003; Marthas and others 2001) has shown that colonies of Chinese-origin rhesus macaques may be of significant utility for AIDS-related research, despite differences from rhesus macaques in immune response to simian immunodeficiency virus.

GENETIC VARIATION WITHIN BREEDING POPULATIONS

The central goals of genetic management are to maintain genetic variability and to avoid inbreeding in order to maximize the long-term viability of captive populations. Achieving the goal of maintaining genetic variability obviously requires that colony managers be able to assess genetic variability. Genetic marker data for a large number of loci provide the most direct means of assessing genetic variability and can be used to estimate and monitor levels of heterozygosity in the population (e.g., Morin and others 1997). When genetic marker data are unavailable but the pedigree is known, genetic variability can be assessed from estimates of the genetic variance in phenotypic traits (such as clinical chemical and hematological traits), which are routinely included in animal colony records (Williams-Blangero and others 1993; 1994). Alternatively, information about the existing pedigree structure alone may be used to estimate genetic variability using computer simulation approaches (e.g., Caballero and Toro 2000; Dyke and others 1990; MacCluer and others 1986).

Genetic variability can be maintained by maximizing the effective population size, a process that minimizes the rate of loss of rare alleles (Kimura and Ohta 1969). The effective population size is essentially the breeding portion of the population, which can be increased by equalizing the genetic contributions of founder animals to the population. For example, the chimpanzee colony at the Southwest Foundation relied on a relatively small proportion of potential sires as breeders, resulting in a large variance in male reproduction (Williams-Blangero and others 1992). A computer simulation experiment demonstrated that if sires had been

randomly selected from the pool of available sires that were of breeding age and were unrelated to the dam, the effective population size of this colony would have almost doubled (Williams-Blangero and others 1992).

Inbreeding (mating between related individuals) can result in the loss of genetic variability and accumulation of deleterious recessive alleles. In nonhuman primates, inbreeding has been shown to increase morbidity and mortality and to decrease reproductive performance (Crawford and O'Rourke 1978; Noble and others 1990; Ralls and Ballou 1982). Colony managers should select unrelated mate pairs to avoid these effects of inbreeding depression. The cumulative effects of random inbreeding can be minimized by equalizing the sex ratio of breeders, maintaining genetic representation of founder animals, and bringing new unrelated animals into a colony (Williams-Blangero and others 2002).

PEDIGREE CONSTRUCTION, VERIFICATION, AND MANAGEMENT

The pedigree structure of a colony is the fundamental piece of information required for effective genetic management of the population, for selection of samples of unrelated experimental animals, and for genetic epidemiological research. Detailed colony records are essential for pedigree reconstruction. If single-male breeding groups are used, colony records may be sufficient to enable pedigree construction for genetic management purposes and for quantitative genetic analyses. Extended pedigrees can be reconstructed from colony record information on the individual's identification number, the dam's identification number, and the sire's identification number for each colony animal (providing that each animal has a unique identification number) utilizing PEDSYS, a pedigree-based data management system (Dyke 1989).

However, it has long been recognized that errors in caging records and assignment of paternity and of maternity can occur even in the most carefully managed colonies (Curie-Cohen and others 1983; VandeBerg and others 1990a). Genetic marker information can be used to verify pedigrees constructed from colony records when paternity is thought to be known (e.g., VandeBerg and others 1990a). If multimale breeding groups are used, pedigrees can be reconstructed using genetic marker data provided that all of the potential sires for a given offspring can be identified and evaluated for mendelian consistency with the genotypes of the dam and the offspring. Pedigree reconstruction by exclusion of all but one potential sire for a dam-offspring pair has been successful in establishing pedigrees for multimale breeding groups of chimpanzees, rhesus monkeys, and vervets (Ely and others 1998; Newman and others 2002; Smith 1980; Vigilant and others 2001).

INCREASING THE VALUE OF PEDIGREED NONHUMAN PRIMATES FOR GENETIC RESEARCH

Pedigreed colonies of nonhuman primates are extremely valuable for genetic research. The statistical power of a genetic epidemiological study is a function of the size and complexity of the pedigrees included in the analyses (Almasy and Blangero 2000). Nonhuman primate colonies frequently have complex extended pedigrees that can be used in quantitative genetic analyses of variation in normal and disease-related phenotypes for both biomedical research and management purposes.

Generation of detailed genotypic information for extended nonhuman primate pedigrees as part of a genome scan is the ultimate way to increase their value for biomedical research. Provided that the families were not originally selected on the basis of a disease trait, a genome scan sample can be used for genetic investigations of any disease-related or normal trait that can be characterized in the population. A genome scan utilizes information on large numbers of markers spaced evenly throughout the genome in conjunction with a map of the markers to chromosomal locations and disease-related trait information, to localize the individual genes influencing the trait to specific chromosomal regions. The sample of baboons at the Southwest Foundation originally selected for a genome scan for traits related to osteoporosis has subsequently been used to localize and characterize genes influencing cardiovascular disease, hypertension, and levels of reproductive hormones (Kammerer and others 2001; Mahaney and others 1997, 1999b; Martin and others 2001a,b).

NONGENETIC INFORMATION REQUIRED TO IMPROVE THE VALUE OF PEDIGREED NONHUMAN PRIMATES

Detailed management and disease histories for pedigreed individuals can be invaluable for future genetic analyses. Management information, such as rearing history, may be critical in the evaluation of phenotypic traits. For example, breast feeding and formula feeding are known to have differential effects on thyroid hormone levels in infant baboons (Lewis and others 1993). Nursery rearing was a significant covariate in genetic analyses of lipoprotein variation in the Southwest Foundation's pedigreed baboon colony (Mahaney and others 1993). Detailed animal histories enable the explicit evaluation of covariate effects, which may improve phenotypic characterization and consequently the power of genetic analysis.

Veterinary records of naturally occurring diseases may provide a rich resource for identifying new disease-related phenotypes for genetic analysis. The phenotypic data on risk factors for disease included in medical

records can facilitate preliminary analyses of genetic effects that can then be used to justify a full-scale genetic study of a given phenotype.

CONCLUSION

Genetic management is critical for the effective management of nonhuman primate colonies and for advances in nonhuman primate genetic research. To maximize the utility of nonhuman primate colonies for genetic research, it is imperative to maintain detailed pedigree information, clinical histories, and management records. The development of new gene maps for nonhuman primates will be essential for linkage analyses designed to localize disease genes in species other than baboons. Increased genotyping is needed to facilitate pedigree verification/reconstruction and future linkage analyses in existing captive nonhuman primate populations.

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Influence of MHC Gene Products on Immune Control of AIDS Virus Infection: Consideration for Use in Nonhuman-Primate Resources

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The global HIV pandemic is now entering its third decade. As of late 2000, there were more than 35 million people infected with HIV worldwide, with almost 70% of infected individuals residing in Sub-Saharan Africa. Although there have been extraordinary recent advances in treatment of HIV infection, the pharmaceuticals that mediate these treatments remain available only to a small percentage of the world's individuals infected with HIV. Indeed, the burden of infection is borne predominantly by developing nations in which access to antiviral drugs and in-depth clinical care is negligible. Therefore, there remains an urgent need to develop an effective prophylactic vaccine against this virus.

Crucial insights about AIDS virus pathogenesis and vaccine efficacy have come from the simian immunodeficiency virus-infected rhesus macaque, the best available animal model for HIV infection of humans. There is every reason to believe that this model will continue to provide a basis for fundamental understanding of virus-host interactions in HIV. Recent results from our laboratory and others have shown that the major histocompatibility complex (MHC) class I genotype can have a dramatic effect on immune control of infection with highly pathogenic immunodeficiency viruses. Moreover, in recent years, several groups have made important contributions to our understanding of the properties of Mamu-

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A*01, a common MHC class I molecule responsible for some macaques' relative ability to control AIDS virus infection. However, as a result of the careful analysis of Mamu-A*01 and the cellular immune responses it restricts, there is now an acute shortage of macaques expressing this molecule available for further study. Efforts to understand the influence of immunogenetics on AIDS in the macaque model must therefore be broadened to include other common MHC class I and class II alleles. This understanding will not only increase the power of the macaque as a model for HIV disease but will also ensure that valuable animal resources are not depleted.

NATURAL HISTORY OF HIV/SIV INFECTION

Although the human and simian immunodeficiency viruses have been the subjects of intense experimental scrutiny since their discovery in the mid-1980s, little is known with certainty about the mechanisms of disease associated with these pathogens. In most individuals, the natural history of immunodeficiency virus infection is a triphasic process. Primary infection is characterized by a rapid burst of viral replication, with virus titers in both HIV-infected humans and SIV-infected macaques reaching higher than 10^7 RNA genome equivalents (copies) per milliliter plasma. Resolution of acute-phase viremia occurs within several weeks of infection, initiating a chronic phase, which can last from a few months to several years. This chronic phase is thought to represent a dynamic equilibrium, in which a balance is struck between ongoing viral replication and its partial control by the immune response, resulting in a steady-state or "set-point" in which virus burdens remain essentially stable. Finally, almost all infected individuals succumb to the infection, showing an increase in virus load and a precipitous loss of $CD4^+$ T cells, resulting in an increased susceptibility to opportunistic infections.

ROLE OF CTLs IN THE IMMUNE RESPONSE TO HIV/SIV

The eventual death of most individuals infected with immunodeficiency viruses stands in apparent contradiction to the observation of strong humoral and cellular immune responses in most infected hosts. Recent advances in technology have greatly facilitated the study of the antiviral CTL response both in HIV-infected humans and in the SIV-infected macaque. Together, the many recent studies of CTL activity in immunodeficiency virus infection have suggested that CTLs play a major role in the modulation of the HIV- and SIV-associated disease course.

Because CTLs are apparently responsible for controlling virus replication in the acute phase, and because the value of the viral load at set-

point strongly predicts the rate of disease progression, the strength of acute-phase CTL responses may "set the set-point" and largely determine disease outcome. Robust CTL responses present during the acute phase would then appear crucial, and indeed possibly sufficient, for control of infection. However, most infected individuals do progress to AIDS, despite vigorous CTL responses. One is then presented with another apparent contradiction: How can these viruses persist in a host in the face of a strong cellular immune response? Effective CTL responses likely exert selective pressure on these notoriously mutable pathogens. Could they vary their antigenic properties in such a way as to avoid immune detection? In fact, there is now overwhelming evidence that HIV, SIV, and other viruses are capable of spawning variants that allow them to "escape" from specific CTL responses, and furthermore that this escape can occur within the first weeks of infection.

IMMUNODOMINANCE OF SIV-SPECIFIC CTL AND CONTROL OF SIV INFECTION

Most studies of CTL activity against SIV in the rhesus macaque have centered on responses restricted by a single common MHC class I molecule, Mamu-A*01, expressed in ~20% of captive-bred macaques of Indian origin. For example, we characterized 14 epitopes derived from SIVmac239 bound by Mamu-A*01 recognized during chronic infection. Of these, CTL directed against the Gag CM9 epitope were present at the highest frequency, and were therefore considered immunodominant. In other studies, we determined that CTL specific for another epitope, Tat SL8, were present during acute infection at frequencies equal to those of Gag CM9-specific CTL. Tat SL8- and Gag CM9-specific CTL are thus equally immunodominant during the acute phase. In fact, CTL of these two specificities were the most frequent in all Mamu-A*01-positive macaques assayed, regardless of the other MHC class I molecules each animal expressed. Mamu-A*01-restricted CTL may thus reproducibly be dominant to other CTL responses in every Mamu-A*01-positive animal, irrespective of the MHC context in which Mamu-A*01 is expressed.

Immunodominance relationships are determined simply by enumerating the frequencies of antigen-specific CTL. Several recent technological innovations have simplified this task enormously; however, although it is now much less technically demanding to quantify antigen-specific CTL, the physiological importance of immunodominance relationships remains unclear. In the case of AIDS disease and pathogenesis, one might wish to determine which CTL responses are the most effective, that is, which responses are best able to eliminate virus-infected cells. These immune responses would make attractive candidates for targets of future vac-

cines. The relationship between Tat SL8- and Gag CM9-specific CTL responses mentioned above may provide insights into ways of determining CTL efficacy, and it also may demonstrate the utility of the SIV-macaque model to the fields of AIDS pathogenesis and vaccine design.

"FUNCTIONAL AVIDITY" OF CTL AND SELECTION FOR ESCAPE MUTANTS

Faced with the observation that CTL specific for two Mamu-A*01-restricted epitopes appeared to be equally immunodominant during acute SIV infection, we sought to determine whether any differences exist in the impact of CTLs with these specificities on the virus population. As we had previously shown, CTLs that recognize the Tat SL8 epitope rapidly and reproducibly select for viral escape variants within 4 weeks of infection with SIVmac239. Conversely, although we and others have observed mutations within the Gag CM9 epitope that are consistent with CTL escape, these mutations appear in the viral population much later, beginning around 1 year after infection. Moreover, mutations in the Gag CM9 epitope do not appear to occur reproducibly in all Mamu-A*01-positive macaques. Because CTLs recognize these two epitopes exist in relatively equal frequencies during the acute phase of infection in most Mamu-A*01-positive animals, we reasoned that immunodominance alone cannot account for the differential ability of CTLs to eliminate susceptible viruses from the actively replicating population. Tat SL8-specific CTLs appear rapidly able to eliminate susceptible viruses, such that viruses with wild-type epitope sequences are not detected in Mamu-A*01-positive animals by 6 weeks after infection.

We hypothesize that this phenomenon can be understood in the following manner: Replication of the infecting (wild-type) virus population is reduced by an effective acute-phase CTL response. Rapid viral turnover then leads to a replacement of the wild-type sequence by mutant viruses, which have "escaped" this CTL response. The steady-state virus population measured during chronic infection would then represent mutants that have escaped the most effective CTL response. If this model is true, we would expect that the more rapidly the wild-type virus is eliminated, the more effectively the infection is controlled inasmuch as viral replication is contained more rapidly, allowing less time for the generation of escape mutants.

What mechanisms can account for the differential ability of CTLs to select for escape variants with rapid kinetics? Other investigators have shown that some CTLs require a lower density of MHC class I-peptide complexes on the surface of target cells to be sensitized to perform their effector functions. CTLs that are sensitized at relatively low peptide con-

centrations were dubbed high "functional avidity" CTLs. It was proposed that these cells may be particularly effective at eliminating virus-infected targets because they could recognize these targets early in the infection process, when cell surface antigen densities were low.

We therefore determined whether CTLs detected in our SIVmac239-infected animals showed differences in "functional avidity." We used titrations of epitope peptide (conc. 0.01 – 10000 n*M*) in interferon-gamma (IFN-γ) intracellular cytokine staining assays of fresh peripheral blood mononuclear cells to determine (1) the maximum IFN-γ output of CTLs recognizing different SIV-derived epitopes, and (2) the peptide concentration that sensitized these CTLs to release half the possible maximum of IFN-γ. CTLs with low half-maximum concentrations were therefore considered to be of high "functional avidity," and CTLs requiring high peptide concentrations to reach half-maximum IFN-γ output were considered of low "functional avidity." Strikingly, we found that Gag CM9-specific CTL required ~20 n*M* peptide, whereas Tat SL8-specific CTL required only ~0.1 n*M* peptide. We therefore conclude that Tat SL8-specific CTL demonstrate very high "functional avidity." It is possible that this high sensitivity to their cognate epitope allows Tat SL8-specific CTLs to detect infected cells very early in the viral replication cycle that have only small densities of the Tat SL8 epitope on their surfaces, before the production of progeny virus particles. This explanation would help to account for the extremely rapid turnover of the viral population observed during acute infection in Mamu-A*01-positive animals.

SUMMARY AND RECOMMENDATIONS

The SIV-infected rhesus macaque remains the best available animal model for HIV infection of humans. Many important and incisive studies are feasible in macaques but would be impossible in humans. For example, our studies of viral evolution and the cellular immune response are predicated on our exact knowledge of the time, route, and dose of virus infecting the animals. Most importantly, we are able to challenge macaques with a pathogenic, molecularly cloned virus. This technique allows us to compare sequences of viral isolates obtained after infection with an inoculum that is both clonal and well defined, permitting us to dissect viral variation and evolution much more finely than is possible in human subjects. Knowledge of the infecting virus also allows us to design peptide reagents for cellular immune assays that reflect accurately the viral sequences present in infected animals, and limits the variation among experimental subjects in detection of these responses that is inevitable when using peptides derived from consensus HIV sequences on patient samples.

Moreover, fine analysis of selection on viral populations mediated by cellular immune responses also requires precise definition of viral epitopes, so that viral variation within and without epitope sequences can be accurately recognized. Unfortunately, this area of SIV research in macaques has, until recently, been relatively neglected. Complete data on peptide binding motifs are available for only a handful of macaque MHC class I molecules, and the SIV genome has been searched exhaustively for epitopes bound by only one molecule, Mamu-A*01, discussed above. Therefore, most studies of SIV-specific CTLs in macaques have hitherto focused on those responses restricted by Mamu-A*01, for which the most complete data were available. There is accordingly an acute shortage of Mamu-A*01-positive macaques available for research. We must use these valuable animal resources wisely and parsimoniously, to ensure their future availability.

One key way in which we can improve the macaque model of AIDS is to increase its similarity to the human clinical picture. To date, mucosal challenges with SIV have been carried out with large boluses of the virus so that all control macaques become infected after only one exposure. This system may overwhelm a potentially protective immune response induced by vaccination. In humans, it is thought that approximately 250 to 1000 unprotected sexual encounters with HIV-infected men are needed to cause HIV infection. There is thus a critical need to develop a low-dose intravaginal challenge model in the rhesus macaque. The shortage of female Indian macaques precludes this as a realistic goal for macaques bred in the primate center system. To develop a more physiologically relevant intravaginal challenge model, we will, therefore, import female macaques from China to initiate such studies in our primate center. We hope that these studies, and similar ones beginning at other primate centers, will help to develop and extend the utility of the rhesus macaque model for AIDS.

Indian- and Chinese-origin Rhesus Macaques for AIDS-related Research: Comparison of Vaginal Transmission Efficiency of Simian Immunodeficiency Virus (SIV), Viral Loads, and Virus-specific Antibody Responses

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INTRODUCTION

The use of rhesus macaques as a nonhuman primate model for human HIV infection and AIDS has resulted in an unprecedented demand that has far exceeded the supply of domestically bred animals; thus, researchers must use monkeys from other sources. Most domestically bred rhesus macaques are derived from animals imported from India. Because Indian macaques can no longer be exported, China has become one of the most reliable sources for rhesus macaques. However, it has been reported that the clinical course of SIV infection is slower and more variable in Chinese-origin monkeys compared with Indian origin monkeys (Joag and others 1994). We designed a study to determine whether Chinese-origin rhesus monkeys are more resistant to infection after intravaginal (IVAG) SIV inoculation compared with Indian-origin rhesus macaques (Marthas and others 2001). The findings of this recently published study are summarized below.

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RESULTS AND DISCUSSION

We found no significant difference in the number of animals infected after one or two IVAG inoculations for Indian-origin compared with Chinese-origin macaques. Thus, rhesus monkeys originating from both countries are useful for studies requiring SIV transmission and infection. However, consistent with results of our previous studies, two IVAG doses of SIV resulted in significantly more SIV-infected macaques than one IVAG inoculation (Miller and others 1990, 1992).

We also compared the level of viremia in SIV infection in Chinese- and Indian-origin rhesus monkeys during the first few weeks of infection. As previously reported, SIV RNA levels in plasma among SIV-infected macaques were variable, and the variation was greater among the Indian-origin than among the Chinese-origin rhesus monkeys. SIV RNA levels at 2 weeks postinfection (PI) in plasma of Chinese- and Indian-origin animals were found to be high but not significantly different. However, by 6 weeks PI, the plasma SIV RNA levels were significantly lower in Chinese- compared with Indian-origin rhesus macaques, despite large overlap in the range of viral loads among Indian- and Chinese-origin animals. Our result is consistent with earlier observations from smaller numbers of Chinese and Indian rhesus macaques inoculated parenterally with SIV (Joag and others 1994).

Anti-SIV plasma antibody levels were also more variable in the Indian-origin rhesus macaques; however, at 6 to 8 weeks PI, there were no significant differences in SIV-antibody titers for Chinese- and Indian-origin rhesus macaques. It is well documented that Indian-origin rhesus monkeys that fail to make an antibody response to SIV or SHIV infection have a rapid disease course (Daniel and others 1987; Kimata and others 1999; Lewis and others 1994; Lu and others 1998). Our study found that rapid progression to AIDS (i.e., within 3 months PI) occurs at similar frequency in SIV-infected rhesus monkeys of Chinese-origin (1 of 10) and Indian-origin (1 of 16).

We used a panel of 13 highly polymorphic microsatellite markers to assess the degree of genetic similarity between monkeys of Chinese and Indian origin. Consistent with expectations for geographically separate populations of a single polymorphic species, we detected the majority of alleles for the 13 microsatellite loci in both Indian-origin and Chinese-origin animals; however, some allele frequencies differed among Indian- and Chinese-origin animals as reported previously (Morin and others 1997). We found no microsatellite alleles that were diagnostic for Chinese or Indian origin.

Overall, we found that the geographic origin of rhesus macaques does not predict the efficiency of vaginal SIV transmission or the level of

SIV RNA in plasma of SIV-infected animals during the first few weeks after IVAG inoculation. Most importantly, our results demonstrate that both Chinese-origin and Indian-origin rhesus macaques are well suited for AIDS-related studies that require mucosal SIV infection.

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Session 4: Panel Discussion

Participants:
John L. VandeBerg—Session Chair, Southwest Foundation for Biomedical Research, USA
Sarah Williams-Blangero—Southwest Foundation for Biomedical Research, USA
Thomas Friedrich—Wisconsin National Primate Research Center, USA
Marta L. Marthas—California National Primate Research Center, USA

QUESTIONS AND ANSWERS

PARTICIPANT A: Dr. Friedrich, you referred to the similarity in the Mamu system to that in the human in terms of regulation of escape. Could you please give us a little more detail.

DR. FRIEDRICH (Thomas Friedrich, Wisconsin National Primate Research Center): I will give you as much detail as I can. I think Dr. Marthas might like to comment as she alluded to some of the similarities between the human MHC system and the rhesus in her talk.

Basically, at a very simplistic level, there is a high degree of similarity. We do see selection for escape variant viruses by certain specific CTL in the human system. The escape is much more difficult to define in humans because they are infected with a heterogeneous population of viruses. By the time you get a human subject to study, it is usually too late to be able to define the actual genotype of the infecting strain of virus. It is much

more difficult to compare the viral sequences that you isolate from humans with earlier strains and to define this kind of thing.

As opposed to the macaque system, we can knowingly infect them with clonal viruses. That said, as Dr. Marthas discussed, there are certain alleles in the human that are associated with either a susceptibility to a rapid progression in AIDS or a resistance. HLA B-27 and B-57, for example, are associated with slow progression.

There are reports of human long-term nonprogressors, as they call them, which look clinically like the three SIV controllers that I described. They have low virus loads in the chronic phase of infection. They often have antigen-specific CD4 responses, which you would not normally detect. They have CTL responses that you might expect would have selected for escape variance, which they do not seem to have done, and so on. At that level, there is definitely a high degree of similarity between our models of what goes on in HIV-infected humans.

DR. KRAISELBURD (Edmundo Kraiselburd, Department of Microbiology Medical Sciences, Puerto Rico): Congratulations to individuals on the Genetics Panel for an excellent presentation. My question for Dr. Marthas is, do you have any data in terms of the disease preparation in the Chinese (rhesus)? In other words, we know there is a relationship between the nadir; is there progression? I have seen some data for the rhesus macaque, which is a slow progressor.

Second, of the rhesus that we have in Puerto Rico, 20% are Mamu-A*01 positive. I understand that some of your monkeys came from Puerto Rico. I would like you to comment on the fact that we did not see any relationship of the few monkeys that we tested in terms of disease preparation and Mamu-A*01 marker.

DR. MARTHAS (Martha L. Marthas, California National Primate Research Center): For this study, we did not measure long-term progression, which we originally began to titrate, so we purposely ended the study by 6 weeks. However, from other studies, the animals that have the controlled viremia, like the ones Dr. Friedrich described, are expected to continue long term. They have a more prolonged or delayed AIDS. If the animals, for whatever reason, had a high set point or did not control, they would have a more rapid progression to disease.

Dr. Mark's group, which studies Indian versus Chinese, is similar although there are a couple of differences. First, they did an intravenous inoculation, which can make a difference in how the virus affects the animals. Second, they had a small sample size of Indians. If I had picked any two of my animals, I might have obtained a result similar to theirs. Their Chinese results looked very similar to ours initially. I would predict that if they were followed, some of those animals would have been very controlled.

The second question was A1 and why different groups see differences in the way Mamu-A*01 progresses. My hypothesis is that Mamu-A*01 may be a marker for something else. It happens to be convenient. I would predict that based on the human, there are linkages to equilibrium; however, there are associations that are nonrandom between Class 1 and other alleles on that same chromosome. So it might be that Mamu-A*01, from different geographic locations originally (we do not know where all of our Wisconsins or Indians came from) might be associated with something else that is controlling. Our Mamu-A*01 is tracking from Mamu-A*01, but it is not associated with whatever the other important alleles are tracking.

DR. FRIEDRICH: I agree with Dr. Marthas. I told you about our second experiment: we inoculated another set of three Mamu-A*01/Mamu-B*17 double-positive animals. Because our laboratory does a lot of work on CTL responses, we were fervently hoping to find recapitulation of the scenario that took place with our first set of Mamu-A*01/Mamu-B*17 animals. Unfortunately, the animals did not control.

As Dr. Marthas was saying, although certain MHC Class 1 molecules basically may be markers for the ability of these animals to control, and we may be able to understand the ways in which these molecules may contribute to the overall control that we see, we simply do not know enough yet about macaque genetics to identify with certainty the other factors that might be influencing this ability. The experiment we performed recently shows that it is not only two MHC Class 1 alleles which is not surprising but is still disappointing.

DR. ROBERTS (Jeffrey Roberts, University of California at Davis): I have two questions pertaining to issues of population management. First, Dr. VandeBerg, in terms of the desire to maintain large populations of animals for pedigree, does the population at Southwest contribute to the availability of aged animals down the line, both in terms of genetic studies and availability of cost-effective production of aged animals? Second, Dr. VandeBerg or Dr. Williams-Blangero, in large multimale groups particularly at Southwest, are you concerned about having these large populations and about very cost-effective housing? If you have determined that there are males that have not contributed to the gene pool effectively for social reasons or whatever, do you advocate strategies to harvest those males at certain points either for indoor-timed mating or for other means of assisted reproductive technology to maximize their contribution?

DR. VANDEBERG (John VandeBerg, Southwest Foundation for Biomedical Research): With regard to aged animals, indeed, by maintaining these pedigreed animals throughout their lives, clearly we do end up with animals that have reached the end of their useful reproductive careers. We channel them into a specific group that we call the pedigree

geriatric baboon colony. We actually have more than 200 baboons in that colony now that are older than 17 or 18 years. Some are nearly 30 years old! We are developing some new research programs to study the aging process in those animals. They all are pedigreed and genotyped, and they will have been breeders to get to that age. They will have many progeny—grand progeny and perhaps great grand progeny—in the population. They are an extraordinarily valuable resource. It costs nothing to get them into the geriatric colony in the sense that they were productively used for research and breeding throughout their useful lives.

As for equalizing numbers of progeny from breeders, one of the most effective ways to deal with that situation is to harvest selectively. It may not be necessary to equalize the number of progeny of various males or females, but what you harvest for terminal experiments are an unequal number of progeny from particular parents so that you are very careful when you save back your breeders that you have equalized the genetic contributions from your females and from as many males as you can use in the particular breeding scheme. We effectively follow that process with our colony.

DR. WILLIAMS-BLANGERO (Sarah Williams-Blangero, Southwest Foundation for Biomedical Research): For the pedigreed section of the colony, we use all single male breeding.

DR. LYONS (Dr. Leslie A. Lyons, University of California at Davis): Dr. Friedrich, I think Dr. Marthas' point about linkage to equilibrium is extremely valid. Do you know how your monkeys were related in either of your studies?

DR. FRIEDRICH: The monkeys we used for that study were not related to each other. Beyond that, I really cannot tell you. We can find that information in our colony records because they are kept in an animal and sire-dam triplicate, as described in previous talks. There is no easy way for us to go beyond that and determine what other genetic factors might be playing a role in these animals, short of sequencing their entire MHC side and finding out what happened.

DR. LYONS: Along those lines, would you suggest that while we are waiting for other sources of exports or identifying other populations of animals, we could genotype those animals and establish pedigrees so that we could bring in known different varieties of these animals to help our colonies. In other words, should we help those other countries get their animals genetically characterized?

DR. VANDEBERG: Such a plan would need to be carefully constructed with clear goals and a clear understanding of the actual potential for sending those particular animals to this country or to another country for research purposes.

DR. HEARN (John Hearn, Australian National University): First, Drs.

VandeBerg and Williams-Blangero, the issue of pedigreed and genotyped colonies is clearly a major added value that, spread more broadly, can not only give us high quality research and design but can perhaps also reduce the number of animals required in particular questions. Do you think we are at a stage where you could recommend a set of minimal criteria for genetic characterization and management of colonies in general as a separate issue to the kind of in-depth analysis that you would need for each specific disease, that has both cost and practicality connotations?

DR. WILLIAMS-BLANGERO: At a minimum, to begin constructing the pedigree only from the colony records gives you enough information to begin quantitative genetic analysis. With this information, which you can obtain from basic colony records for many nonhuman primate colonies, you can begin to ask the simple question: how much variation in this trait is attributable to genetics? As you ask these questions in conjunction with the existing phenotypic data on normal variation and traits in which you might be interested and on disease traits that are recorded in the clinical records, you can get an idea about productive directions for a true genome scan or more detailed genetic research. I think at whatever level you can feasibly do this, you enhance the value of the colony tremendously by adding any pedigree information. If you have genetic markers that are generated as part of other studies, and can contribute that information back into the colony to use for paternity testing and other purposes, it is of great value for enhancing the colony for genetic research.

DR. VANDEBERG: Let me add to that, however, that there are circumstances in which it is appropriate and cost-effective to produce animals in large breeding groups, such as our corrals or 6-acre breeding corral, which has about 600 baboons in it. I think the answer to your question must be tailored to the breeding situation, so there are situations where it is appropriate to have small breeding groups, large breeding groups, and so forth. Certainly, for genetic research, if it is economically feasible and practical to have single male breeding groups, that arrangement is by far the most valuable for genetic research, especially in the absence of markers to sort out paternity.

I think it would be difficult to give an overall set of minimum recommendations that would fit all breeding situations. I think they will have to be tailored to specific breeding situations and particular breeding objectives.

DR. HEARN: Thank you. My next question concerns emerging diseases—the next AIDS or, in particular, the transmission of viruses or zoonoses between nonhuman primate populations and humans. In captive colonies, or specifically in the wild where around the world there are particular areas where humans and nonhuman primates come into close

contact, and increasingly in areas where there is great ecological pressure, the situation is being set up for potential transmission. Are we ready, or should we be starting to investigate that issue?

DR. MARTHAS: I think it is important to set the boundaries and to let people know about this exposure. Participants at a recent meeting in Keystone, Colorado, were talking about HIV pathogenesis and starting to look at two different things: wild chimpanzees in preserves and SIV isolates. They are finding SIV isolates in chimpanzees using noninvasive methods like fecal and urine sampling. They are able to detect and indeed find these additional SIV isolates in populations that would not have been able to be tested before. By documenting and analysis, they are able to show that there are multiple crossover points from chimpanzees—multiple lineages of what is now an SIV that could potentially then go from chimpanzee to humans.

How did the SIV get into the chimpanzees? To make a long story short again, the answer is probably from other monkeys because chimps are known to hunt and eat other monkey species. That probability was, in fact, documented by a person looking and finding a variety of other species new SIV isolates in a variety of species that had not previously been found before. The more we look, the more we are finding it. There is more potential for human exposure and other nonhuman primates to retroviruses in this case, so we conjecture that it is exposure to any pathogen—parasites or other blood borne transmission.

With respect to your conservation question and human intervention, the most recent evidence is that the way the person sampled the 20 or more species of primates in Cameroon and central Africa was by going to bush meat markets. They sampled meat from monkeys that had been killed for human consumption. While consumption might not be the worst case scenario, certainly the preparing and hunting of those animals exposed the people and then exposed a broader population by eating the animals. I think it is very important, but we do not have an answer for how to deal with it.

DR. FRIEDRICH: Dr. Marthas lead into the current hypothesis for the transmission of HIV into humans. First, HIV began as zoonoses probably through the consumption of chimpanzees in bush meat that was infected with the precursor of HIV. We have this information from the work of Beatrice Hahn and Betty Korber. I do not know what primate centers and investigators of disease in nonhuman primates can do to be prepared for this kind of eventuality. We can only make public these findings so that people understand that by placing themselves and nonhuman primates at risk in these high-pressure ecological environments, it is more likely that we are going to find this sort of transmission occurring in the future. As Dr. Marthas said, the more you look, the more SIV

isolates you find, which is more potential for another zoonoses to occur in humans. In addition, there is every reason to believe there are other viruses we have yet to discover that could do the same thing.

DR. VANDEBERG: I would like to add that I think the primate centers and the primate community in general are actually very poorly prepared to deal with these issues in part because we do not have the facilities that are required. Our biosafety level 3 facilities are woefully inadequate in this country, and I am sure around the world, to deal with those kinds of issues. We do not have any biosafety level 4 laboratories capable of housing living animals. At our primate center, we are turning down studies that require biosafety level 3 facilities including studies on tuberculosis, anthrax, and West Nile virus. We are not meeting the current needs for lack of facilities, and we are certainly not in a position to meet the emerging needs that we do not even know of today.

The base grant budgets for the primate centers have been essentially flat over the 5-year doubling of the NIH budget. We have struggled to maintain what we have, not only in terms of facilities but also in terms of personnel. We are not in a position to recruit the personnel that are needed to establish the critical masses of scientists required for those kinds of investigations.

In regard to the potential of transmission of disease from chimpanzees, as we scale down the number of chimpanzees that are available for biomedical research, it is entirely possible that at some future point there will be another disease emerge, like AIDS or HIV. With the incredibly long generation time of chimpanzees, it would be very difficult to scale up that population if they were needed for research for a disease of that nature.

DR. ROBERTS: I would like to say that as we look at bringing additional primate sources into the United States, if we cannot genetically characterize them, one of the most crucial things is to establish the provenience of those animals. Where are they originating?

When you look at the range of facilities in China, if you can specify the geographic origin of those populations, it helps incredibly in terms of both looking at the genetics of those populations and also developing genetic tools to compare those populations.

DR. VANDEBERG: I would like to reinforce that point. The large variation that Dr. Marthas described in the Chinese rhesus may well be a consequence of geographic origin, a wide variety of geographic origin of animals. We talk about Chinese and Indian rhesus as if each population were homogeneous. Certainly they are not. Chinese rhesus from one part of China might behave very differently in these kinds of experiments than Chinese rhesus from another part of China. Thank you for mentioning that difference.

DR. ERWIN (Dr. Joseph M. Erwin, BIOQUAL and the Foundation for Comparative and Conservation Biology): I want to underscore the value of this morning's presentations, particularly with regard to phenotypic characterization. The goals of genomics and the promise of proteomics cannot be appropriately realized unless there is a phenomic or phenotypic characterization component that parallels them.

We have heard that, fortunately, some of that characterization is going on. The support of partially characterized and pedigreed colonies is absolutely critical. Furthermore, I think there is the potential for some of the circumstances such as Mauritius, where there is a genetically homogeneous population that came from a relatively small number of founders and the St. Kitts African green population. Some of those island populations are essentially the best that we have in regard to inbred populations. We do not know what the potential is for genetic studies from those sources.

I think it helps to recognize that there is potential for working out some of the genetic risk factors and verifying them within the pedigreed colonies, then going to some of these relatively genetically well-defined biogeographic populations and selecting the animals that are appropriate for whatever the target research is. I think it could add some efficiencies, and one could even extend that selection to some of the other introduced populations such as the *Macaca nigra* population on Ba chang, the *Macaca fascicularis* population on Cabaña, the *Macaca fascicularis* population on Angour in Balow, and a number of other populations of this kind that could become tremendously valuable.

We must not neglect the other captive populations that exist but are currently not well supported. I think most of you are aware of a population of 300 chimpanzees that is now available and in need of support. I am trying to help develop such a support effort. So if you are concerned about this as much as I am, please contact me.

PARTICIPANT B: A message to take home is that making a switch to a whole different species would be of enormous consequence to a laboratory—even after establishing all of the norms and different information that we have on them, including the simple subtleties we see between the Chinese- and Indian-derived rhesus that sometimes are of great importance to a study. If you look at the other differences between the Chinese- and Indian-derived rhesus, you have differences in aggressiveness, serotonin, and alcohol consumption in blood chemistries. Those differences could be very relevant. Adding to what Dr. Erwin said, if you have a question on aggression, perhaps you would want to look at the more aggressive species as a good model. It really depends on what your model is in many cases if you are just beginning, but if you were already established, it would be quite difficult to make a switch.

DR. VANDEBERG: I agree it is very difficult to make a switch. I think it is not practical for an individual investigator to make that kind of a switch, particularly in the short term. However, if resources were committed to establish baseline data on some alternative species to rhesus over a period of time and those baseline data were developed and made available, it would be much easier for investigators beginning projects to choose a species other than rhesus. It may be possible for investigators at some point to switch, and it may be necessary if there are no rhesus available to them. However, the individual investigator is not in a position to make that switch by him- or herself. We must have support for developing the baseline data for many physiological characters for some of these other species that are readily available.

DR. PALMOUR (Dr. Roberta Palmour, McGill University): I would like to respond to Dr. Erwin about the monkeys on St. Kitts. First, we do have a pedigreed colony. We have been doing genetic studies within that pedigreed colony, which is quite large now. Second, we have done some work looking at the genetic variability on the island. Although it is certainly the case that the genetic variability is restricted by comparison with African vervets, there is a significant amount of genetic variability even from these 1000 founders. It is not that we have an inbred colony, but we do have a genetically restricted colony. I think one of the important points for the whole field is that by looking at different sources of variability, we will have models of different aspects of human traits. I think this point will be very important. I know Dr. VandeBerg and Dr. Williams-Blangero agree because they too have been doing this kind of work.

PARTICIPANT C: Is there a *Macaca fascicularis* equivalent to Mamu AO*1, and do you have any plans of expanding into cynos as an SIV model?

DR. FRIEDRICH: It depends on the equivalent. If you want to talk about an MHC allele that may or may not be associated with protection or relative control of SIV, it is quite possible. I am not familiar with any *M. fascicularis* data so I cannot tell you for certain. If you want to talk about an allele that would encode a molecule that would bind the same types of peptides or act in the same type of way, I would guess probably not.

DR. MARTHAS: I do not think there has been a systematic study, for instance, in *M. fascicularis* or other species. It could be done. I know from looking generally at a sequence database that there are similar sequences in MHC alleles where they have been studied for baboons, cynos, and rhesus. However, I do not think anyone has systematically gone through and tested them for whether you can obtain functional data or recognition by reagents. It is important to develop reagents that are either species specific or could work across multiple species.

DR. ERWIN: I also have asked several people that question because it

seems to me a very high priority that for African greens from St. Kitts and Mauritian cynos, this kind of work should be done. I appreciate that MHC is difficult to work with, but it seems to be a very high priority in the context of the discussions we are having at this meeting with regard to limited supply of rhesus.

DR. VANDEBERG: I would like to thank everyone in the audience for that wonderful discussion. It was extremely productive and exactly what we had hoped this session would become. Please join me in thanking the presenters.

Session 5

Microbiology

Microbiological Problems in Nonhuman Primates Used in Research

Gary Baskin, DVM

MICROBIOLOGICAL AGENTS

The following microbiological agents are concerns in relation to research with and management of captive nonhuman primates: viruses, bacteria, parasites, and fungi. I will discuss their different types briefly.

Viruses

Simian Retrovirus (SRV), Type D Retrovirus. SRV is a natural infection of many species of macaques, which causes anemia, immunodeficiency, and retroperitoneal fibromatosis. SRV is very difficult to control because animals may be intermittently seropositive and/or viremic and may be asymptomatic for long periods. One must test for both antibody and virus (culture or polymerase chain reaction) at least three times before determining an animal is SRV negative. Even this testing is sometimes insufficient to detect positive monkeys. There are multiple serotypes that complicate testing protocols.

Simian Immunodeficiency Virus (SIV). SIV is a natural infection of many African primates. SIV does not appear to cause disease in its natural hosts, but it may cause an immunodeficiency disease similar to AIDS in

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macaques. It is also potentially zoonotic. Serology and viral isolation may be used, but there are many different isolates from many different primate species, making detection of unknown strains problematic. SIV is not a natural infection of macaques. SIV infects primarily CD4+ T-cells and macrophages.

Simian T-Lymphotrophic Virus (STLV-1). STLV is a natural infection of many Asian and African monkeys. It is apparently nonpathogenic in Asian monkeys but is sometimes associated with leukemia and lymphoma in African species, especially baboons. STLV infects T-cells.

Herpesvirus simiae **(Herpes B, B-virus, Cercopithicine herpesvirus 1).** Herpes B is endemic in most captive macaque colonies where the incidence often approaches 100% in adults. Herpes B is the macaque homologue of herpes simplex I in humans, in that it seldom causes serious disease in macaques (except in *M. radiata* and *M. fascicularis*). The ability of herpes B to cause fatal encephalomyelitis in humans makes the zoonotic potential of this virus its most significant aspect.

Herpes papio. Herpes papio is endemic in many baboon colonies. It is the baboon homologue of herpes simplex II in humans, in that it often affects the external genitalia. The lesions are sometimes serious and may disrupt the breeding capacity of affected individuals.

Lymphocryptovirus. Lymphocryptoviruses are γ-herpes viruses that are endemic in many monkey species. They are homologues of Epstein-Barr virus in humans and have been associated with B-cell lymphomas in immunodeficient macaques. They infect B-cells and various epithelial cells.

Rhadinovirus. Rhadinoviruses are γ-herpes viruses that are homologues of HHV-8 (KSHV) in humans. Some research colonies of rhesus and pigtailed macaques have a high incidence of seropositivity. Rhadinoviruses have been associated with retroperitoneal fibromatosis and lymphoid hyperplasia. They produce an interleukin-6-like protein.

Cytomegalovirus (CMV). CMV is endemic in most research colonies. It is a common opportunistic infection in immunodeficient animals.

Adenovirus. There are numerous adenovirus serotypes endemic in nonhuman primate colonies. Adenoviruses are common opportunistic infections.

B-19-like Parvovirus. This parvovirus is a homologue of B-19 in humans and is endemic in many macaque colonies. It can cause fatal anemia in immunodeficient monkeys.

SV40. SV40 is a papovavirus that is endemic in rhesus monkeys. It is a homologue of JC virus in humans and causes progressive multifocal leukoencephalopathy, pneumonia, and nephritis in immunodeficient monkeys.

Orthoreovirus. Orthoreovirus is endemic in some baboon colonies and can cause encephalitis.

Simian Varicella Virus (SVV). SVV is a herpesvirus that is a homologue of human chicken pox. It causes sporadic outbreaks of generalized disease in several African and Asian species. SVV can be carried latently in ganglia.

Encephalomyocarditis Virus (EMCV). EMCV is a virus of rodents that is endemic in wildlife. It causes myocarditis in several species of nonhuman primates.

Measles. Measles is a human virus to which many species of nonhuman primates are highly susceptible.

Simian Hemorrhagic Fever (SHFV). *Erythrocebus patas* and possibly other African monkeys carry SHFV asymptomatically. It causes highly transmissible hemorrhagic fever in macaques.

Ebola. Asian strains of Ebola-like viruses have been found in feral macaques and have caused epizootics of hemorrhagic fever in captive macaques.

Foamy Viruses. Spumaviruses are endemic in many species of nonhuman primates. They are nonpathogenic, but frequently cause cytopathic effect (CPE) in cell cultures. Spumaviruses are also zoonoses.

Bacteria

Shigella. *Shigella* is endemic in many primate colonies. *Shigella* commonly causes ulcerative, hemorrhagic colitis.

Campylobacter. *Campylobacter* is endemic in many primate colonies and commonly causes proliferative colitis.

Helicobacter. *Helicobacter pylori* and *heilmanni* commonly infect the stomach of macaques, where they cause chronic gastritis.

Yersinia. Rodents and birds carry *Yersinia*. It causes enteritis and septicemia in nonhuman primates.

Moraxella. *Moraxella* is commonly carried in the nasal sinuses of macaques, where it sporadically causes acute hemorrhagic sinusitis.

Streptococcus pneumoniae (Diplococcus). *S. pneumoniae* is commonly carried in the nasal sinuses of many nonhuman primate species. It occasionally causes fibrinopurulent serositis, especially in young monkeys.

Bordetella, Klebsiella, Escherichia coli. These bacteria are common environmental bacteria that sometimes cause pneumonia and other infections in many species of nonhuman primates.

Mycobacteria. *M. tuberculosis* and *bovis* may cause tuberculosis in nonhuman primates and are usually acquired from humans. *M. avium/intracellularae* is a common environmental bacteria that frequently causes

opportunistic infections in immunodeficient animals. *M. leprae* may also spontaneously infect some nonhuman primates.

Parasites

Strongyloides. *Strongyloides* are endemic and impossible to control in animals group housed on dirt.

Cryptosporidium, Trichomonas, Balantidium, and Giardia. These protozoa are endemic in many primate colonies and cause sporadic and opportunistic enteric disease.

Sarcocystis. *Sarcocystis* is commonly found in the muscles of wild-caught monkeys.

Babesia. *Babesia* is endemic in many baboon colonies and may cause anemia in stressed animals.

Plasmodium. Many species of *Plasmodium* are endemic in wild and captive populations of many species of monkeys. Most are quiescent until animals are stressed.

Toxoplasma and Trypanosoma. Both protozoa are endemic in wildlife and occasionally infect outdoor-housed primates.

Fungi

Pneumocystis, Enterocytozoon, and Candida. These common fungi are endemic in most primate colonies and frequently cause opportunistic infections in immunodeficient animals.

Chronic Colitis Syndrome. The most common cause of spontaneous death in captive macaques is the chronic colitis, arthritis, amyloidosis syndrome that causes diarrhea, wasting, and death. The cause is unknown, but the syndrome may result from a dysregulated immune response to bacterial antigens in the gut.

FACTORS THAT CONTRIBUTE TO THE DIFFICULTY OF CONTROL

Documentation

It is sometimes difficult to obtain accurate and complete health reports from the source colonies. Health reports should include all testing that has been done (including the dates and types of tests and the results) as well as all previous research, diseases, treatments, and so forth. Colony health reports that detail all diseases, infections, viruses, bacterial isolations, and parasites within the entire colony are also important. The ro-

dent supply industry provides some excellent examples of these types of detailed health reports.

Skilled Personnel

There is a general shortage of laboratory animal veterinarians and veterinary pathologists with training and experience in primate diseases. Most training programs do not provide much exposure to nonhuman primates. Training programs that focus on a PhD often do not produce trainees with the interest and the hands-on medical and diagnostic knowledge and skills that are needed.

Housing

Monkeys in breeding colonies are usually group housed, often outdoors, which makes the control of infectious diseases very difficult. Many primate facilities do not have an adequate number of small enclosures for separating smaller groups of monkeys that carry or do not carry particular agents.

Testing

Viral testing is very expensive and time consuming. Adequate resources for accomplishing systematic testing (e.g., money, reagents, laboratory capacity, quarantine space, labor) are often not available.

Diseases

Investigators are usually experts in their own field of research, but few are knowledgeable about the natural diseases of nonhuman primates. They sometimes do not insist on monkeys that are free of defined agents because they are not aware that the agents exist and could compromise their research, because the monkeys are not available, or because of the cost. No one knows how the various microbial agents might affect particular types of research. None of these agents would be acceptable in rodents used for research. Monkey studies usually use small numbers of animals. Any factor that contributes to variability makes the interpretation of results problematic, especially in small groups.

Although systems have been developed to eliminate some selected viral infections from colonies, there are still no proven methods for eliminating many other infectious agents, particularly bacteria. Most of these types of agents were eliminated from commercial rodent colonies by caesarian derivation and barrier maintenance. This process would be very

difficult in nonhuman primates due to their reproductive physiology. Monkeys raised in isolation develop abnormal behavior. A caesarian-derived infant would require 4 to 5 years to reach sexual maturity, and then would produce one infant per year. The time, cost, and effort required to produce completely clean monkeys is therefore daunting.

RECOMMENDATIONS

As research funding has increased, with particular emphasis on AIDS, and now bioterrorism, the demand for nonhuman primates for research has steadily increased. Unfortunately, funding to support the infrastructure that is required to produce the monkeys has not increased. We are now in the entirely predictable situation that there are not nearly enough animals available for the research that needs to be done. The same level of *intellectual* and *financial* commitment that is currently being applied to research also needs to be applied to the *infrastructure* that supports that research.

Some obvious solutions to this problem are to (1) make better use of the available monkeys by rigorous study design, (2) conserve available monkeys by improving disease definition and control, and (3) increase the supply of monkeys by developing new sources and expanding breeding capacity.

Additional funding to support research into the pathogenesis, epidemiology, treatment, and control of common infectious diseases of nonhuman primates would be very useful. Providing funds for adequate disease surveillance in breeding colonies (necropsy, serology, virology, bacteriology) is important. Additional training programs in laboratory animal medicine and veterinary pathology that emphasize nonhuman primates are needed. Additional emphasis on training of the husbandry staff that cares for nonhuman primates should be given as well. Funds to support the breeding infrastructure, such as corrals, indoor enclosures, clinics, and associated facilities, are needed. All of these activities are very labor intensive and require a knowledgeable, well-trained, and well-supervised technical staff. Guidelines for animal allocation based on prioritized research needs would be very helpful.

Nonhuman Primate Importation and Quarantine: United States, 1981-2001

Tom DeMarcus, MS

In this presentation, I will review the importation and quarantine of nonhuman primates (NHPs) into the United States during the previous 21 years as one component of the international perspective on the future of NHP resources. Specifically, I will (1) describe the US Centers for Disease Control and Prevention's (CDC's) NHP import and quarantine requirements; (2) review the CDC NHP import quarantine program activities for fiscal year 2001 (FY01; October 2000 through September 2001); (3) provide US Fish and Wildlife Service (USFWS) NHP import data for 1981 through 2000 (USFWS 2002); and, (4) describe microbiological and transportation issues related to the importation and quarantine of NHPs in the United States.

CDC NHP IMPORT AND QUARANTINE REQUIREMENTS

To prevent the introduction and spread of communicable diseases capable of causing serious outbreaks in humans (particularly monkeypox, yellow fever, Marburg/Ebola disease, and tuberculosis), importers of NHPs must (Foreign Quarantine Regulations 1985)

Division of Global Migration and Quarantine, National Center for Infectious Diseases, Centers for Disease Control and Prevention, US Department of Health and Human Services, Atlanta, Georgia

1. Register with CDC;
2. Certify that imported NHPs will be used only for bona fide science, education, or exhibition;
3. Implement disease control measures to minimize human exposure to the animals during transportation, isolation, and quarantine;
4. Isolate each shipment of imported NHPs for 31 days, monitor the animals for illness, test them for tuberculosis, test them for filovirus infection (if necessary), and maintain records regarding illness and death;
5. Report suspected zoonotic illness to CDC; and
6. Maintain records regarding the distribution of each shipment.

Before registration and periodically thereafter, CDC inspects importer facilities and reviews their procedures and record-keeping systems. In addition, CDC

1. Reviews proposed plans for each shipment itinerary;
2. Monitors handling of arriving shipments at the ports of entry and at the quarantine facilities;
3. Assesses transportation and disease control measures;
4. Reviews animal health records; and
5. Investigates reports of zoonotic illness.

CDC NHP IMPORT QUARANTINE PROGRAM ACTIVITIES FOR FY01

In FY01, 28 facilities were registered with CDC as importers of NHPs. Registrants included seven commercial importers, six zoos, four regional primate research centers, five universities, five private research facilities, and one pharmaceutical company. These facilities imported a total of 14,710 NHPs in 133 shipments, including

1. 11,915 cynomolgus macaques (81% of total), in 89 shipments;
2. 1867 rhesus macaques (13% of total), in 21 shipments;
3. 456 marmosets (< 3% of total), in three shipments;
4. 135 owl monkeys (< 1% of total), in three shipments;
5. 120 baboons (< 1% of total), in three shipments;
6. 113 African green monkeys (< 1% of total), in three shipments;
7. 44 squirrel monkeys (< 1% of total), in one shipment;
8. 40 pigtail macaques (< 1% of total), in one shipment; and
9. 20 various other species (< 1% of total), in nine shipments.

Cynomolgus, rhesus, and pigtail macaques constituted 94% (13,822 animals) of the NHPs imported into the United States in FY01, including

1. 2766 cynomolgus and 1855 rhesus macaques from China;
2. 3440 cynomolgus macaques from Mauritius;
3. 3120 cynomolgus macaques from Vietnam;
4. 1653 cynomolgus and 40 pigtail macaques from Indonesia;
5. 900 cynomolgus macaques from the Philippines; and
6. 36 cynomolgus and 12 rhesus macaques from Canada.

Other NHP species imported into the United States in FY01 included

1. 451 marmosets from the United Kingdom and five from Brazil;
2. 135 owl monkeys and 44 squirrel monkeys from Peru;
3. 120 baboons from Tanzania; and
4. 89 African green monkeys from St. Kitts and 24 from Tanzania (CDC 2002).

Historically, mortality during transit and quarantine among NHPs imported into the United States has been reported to be as high as 20% (CDC 1989). Since 1990, imported NHP mortality rates have been comparatively low. Of the 14,710 NHPs imported in 133 shipments in FY01, a total of 107 (0.7%) animals in 41 shipments were reported to have died during transit or quarantine. Eighteen (0.12%) of these animals in four shipments were reported dead on arrival at the port of entry. Also during FY01, a total of 526 (3.5%) animals in 38 shipments were reported to have exhibited signs of illness (predominantly stress, diarrhea, or bloody diarrhea) during transit or quarantine. None of these dead or ill animals were reported to have been positive for filovirus antibody or antigen. All 14,710 NHPs imported during FY01 were tested for tuberculosis (TB). A total of 28 (0.19%) individual animals, in seven shipments arriving from five countries were identified as TB suspects. One shipment had 16 suspect animals, one had seven, and five had one each. Five of the seven shipments had positive laboratory reports (histology, culture, polymerase chain reaction, or a combination of these) for at least one animal.

USFWS NHP IMPORT: 1981-2000

This presentation includes a series of 11 charts presenting available USFWS NHP import data for 1981 through 2000 to illustrate trends in US NHP importation for the period. (Space herein does not permit inclusion of these charts. Electronic copies of the slides for this presentation, including the charts, have been provided to the Institute for Laboratory Animal Research [ILAR].) USFWS data for 1993 were not available. CDC FY93 NHP import data for three major NHP species (cynomolgus, rhesus, and African green monkeys) were inserted into the charts in an effort to ap-

proximate continuity in the trends for those species. No CDC FY93 data were available for other NHP species, so that the charts for these species indicate zero imports for 1993.

Some highlights from the combined USFWS and CDC data for the years 1981 through 2001 include the following:

1. Total US NHP imports averaged 13,276 per year for the 20-year period; 16,731 per year for the period 1981 through 1990; and 9821 per year for the period 1991 through 2000. A total of 14,710 were imported in FY01. An upward trend in total NHP imports has emerged since 1998.
2. Total US cynomolgus macaque imports averaged 9335 per year for the 20-year period; 10,857 per year for the period 1981 through 1990; and 7813 per year for the period 1991 through 2000. A total of 11,915 were imported in FY01.
3. Total US rhesus macaque imports averaged 725 per year for the 20-year period; 589 per year for the period 1981 through 1990; and 862 per year for the period 1991 through 2000. A total of 1795 were imported in FY01.
4. Total US African green monkey imports averaged 554 per year for the 20-year period; 937 per year for the period 1981 through 1990; and 171 per year for the period 1991 through 2000. A total of 113 were imported in FY01.

MICROBIOLOGICAL ISSUES RELATED TO NHP IMPORTATION AND QUARANTINE IN THE UNITED STATES

Microbiological issues related to NHP importation and quarantine in the United States include monkeypox, yellow fever, Marburg/Ebola disease, and tuberculosis because of their potential to cause outbreaks of serious diseases in humans. Laboratory testing and health documentation before importation is not a requirement. Rather, importers are required to handle animals as though they are infectious during transportation and through completion of import quarantine. Importers are required to monitor imported NHPs for these and other zoonotic diseases and to report suspected cases among animals or workers.

Importers of cynomolgus, rhesus, and African green monkeys are required to perform laboratory tests for filovirus antigen on animals that die during quarantine. In addition, importers of those species are required to perform laboratory tests for filovirus antibodies on animals that are ill during quarantine with symptoms suggestive of filovirus infection. On rare occasions when positive animals have been found (1989, 1990, and 1996), CDC has worked closely with the importers to implement control measures.

Importers are required to screen all imported NHPs for tuberculosis. Each imported NHP must complete at least three negative tuberculosis skin tests at not less than 2-week intervals, using mammalian old tuberculin licensed by the US Department of Agriculture, in accordance with ILAR standards. When animals are found to be positive or suspect for TB, all remaining animals in the shipment must complete at least five additional negative TB skin tests at not less than 2-week intervals after removal of the suspect animal. This process of isolation and repeated testing is an inexact science and does not always prevent the introduction of TB infection into an NHP colony or into a group of animals that are part of an ongoing study protocol. Such introductions can cause significant morbidity and mortality that can have devastating effects on both the NHP resources and the scientific studies. Promising new TB screening tools to improve the process are being investigated.

Upon completion of quarantine, CDC reviews the veterinary medical report on each shipment and, if appropriate, concurs with its release. CDC may require extension of the quarantine period pending resolution of zoonotic illness issues.

TRANSPORTATION ISSUES RELATED TO THE IMPORTATION OF NHPs INTO THE UNITED STATES

A major transportation issue related to the importation and quarantine of NHPs in the United States is the limited number of international airlines that will transport NHPs to the United States. Before 1990, NHPs were transported to the United States on numerous international airlines; however, since 1990, the number of international airlines that will carry NHP to the United States has been declining. Currently, no major US airlines will carry NHPs to the United States. The number of foreign international airlines that will carry NHPs to the United States is limited.

A number of factors might have contributed to the decline in the number of international airlines that will carry NHPs to the United States. One factor is concern about human health. Although there have been no reported cases of serious illness in humans associated with exposure to imported NHPs in international transit, the potential for exposure is clear. In 1990, CDC required importers to implement disease control measures to minimize exposure to imported NHPs during transit. Before the implementation of these requirements, importers had little direct involvement in the handling of NHPs during international transit. The CDC requirements focused airline attention on NHP shipments.

Another factor is increased emphasis on the International Air Transportation Association regulations. Since 1990, importers and the USFWS have placed increased emphasis on ensuring that NHP shipments comply

with these requirements. Other factors contributing to the decline might include publicity surrounding human Ebola outbreaks (in Africa); human B virus cases (in the United States); and books, newspapers, magazines, media articles, and movies on these subjects. Airlines have also reported that cargo handler unions have made NHP cargo a safety issue in contract negotiations. Finally, animal activists have initiated a letter writing campaign to pressure international airlines not to carry NHPs. It is also important to note that shipment of NHPs represent a tiny fraction of the total volume of international air cargo.

The impact of the limited number of international airlines that will carry NHPs to the United States is significant. For example,

1. Available flights more often require more stops en route, increasing air transport distance, time, and crate handling.
2. Available flights more often arrive at airports distant from quarantine facilities, increasing ground transport distance, time, and crate handling.
3. Longer flight itineraries might increase shipping stress on animals.
4. Available flights are more likely to require aircraft changes en route, increasing shipping stress on animals and requiring more handling of shipping crates.
5. The necessity for more, expensive charters is increased.

CONCLUSION

Information regarding the importation and quarantine of NHPs into the United States over the previous 21 years offers one component of the international perspective on the future of NHP resources. CDC implements U.S. NHP quarantine requirements to prevent the introduction of human disease. In FY01, nearly 15,000 NHPs were imported in 133 shipments; 94% were macaques. The USFWS maintains official NHP import data, which show that total US NHP imports were fewer in the 1990s than in the 1980s, although an upward trend appears to have emerged since 1998. CDC places microbiological emphasis on preventing zoonotic disease capable of causing serious outbreaks in humans. TB has the potential to also affect US NHP colonies and research. The limited number of US and international airlines that will carry NHP to the United States is a major transportation issue.

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Diagnosis of Tuberculosis in Nonhuman Primates

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Tuberculosis (TB) is of great importance in humans and monkeys. In humans, approximately one third of the world's population is infected. Eight million people are infected annually with *Mycobacterium tuberculosis*, and two million die from TB each year (Mustafa 2001). There are limitations to current nonhuman primate, guinea pig, and rodent animal models, and there is continued need for research into preventives and therapeutics.

TB is also very important in nonhuman primates. Unfortunately, necropsy of an acutely dead nonhuman primate all too frequently reveals grossly visible miliary or granulomatous lesions consistent with disseminated TB. Special staining of lesioned tissue then highlights the presence of the causative acid-fast organisms. Much of the information we have on TB in nonhuman primates is anecdotal—verbal reports on what has occurred in colleagues' facilities. There is a comparative paucity of published literature on TB in nonhuman primates, and the information is often contradictory. Some reports outline the diagnostic value of the enzyme-linked immunosorbent assay (ELISA), lymphocyte stimulation tests, and erythrocyte sedimentation rates; and other reports conclude that they are of no value. The same confusing picture exists regarding the predictive value of the polymerase chain reaction (PCR) technique, radi-

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ography, and the classic intradermal test. From the clinical reports that exist primarily for macaques, it is difficult to extrapolate to other species regarding the course of infection and value of diagnostic tests.

In an effort to clarify the conflicting information, we completed a study to evaluate commonly used TB diagnostics. The objectives of our study were to determine the efficacy and predictability of current methods for diagnosis of TB in three species of cercopithecines and to follow the course of the disease in species in which little is known regarding susceptibility to infection. Six cynomolgus, six rhesus, and five vervet monkeys were quarantined and extensively prescreened for the absence of TB infection prior being placed on study. The research was approved by the Institutional Animal Care and Use Committee and conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (NRC 1996). Animals were given intrathecal injection of 100 colony forming units of Erdman strain (35801 obtained from the American Type Culture Collection) *M. tuberculosis*, and studies were conducted under Biosafety Level 3 conditions.

Animals were monitored daily for behavior, appetite, and general health; and any clinical signs such as coughing were noted. Intradermal tuberculin testing was conducted every 2 weeks using mammalian old tuberculin (MOT) in the palpebrum and abdomen and purified protein derivative (PPD) in the abdomen. Animals were radiographed monthly. Tracheal washes were analyzed by culture and PCR every 2 weeks for the first month and then monthly thereafter. Blood was collected every other week, and hematology, clinical chemistries, ELISA, and lymphocyte proliferation assays were performed. The animals had complete physical examinations performed and body weights recorded every 2 weeks. Animals were euthanized at the predetermined study end of 7 1/2 months postinoculation (PI) or earlier, due to pre-established euthanasia guidelines including persistent clinical signs such as coughing, anorexia, or weight loss. A complete necropsy with culture, PCR, and histopathological analysis of collected tissue was performed on each animal at the conclusion of the study.

Surprisingly, vervets were exquisitely sensitive to this experimental TB infection, with all five being euthanized at days 44 to 52 PI. Rhesus monkeys had a variable course of disease, with animals euthanized at 2, 4, 5, and 6 months PI and at study termination. Cynomolgus displayed a more chronic duration of infection, with two animals euthanized at 5 1/2 and 7 months, respectively, and the other four at study termination. On necropsy, all animals had gross evidence of tuberculosis. *M. tuberculosis* infection was confirmed postmortem in all animals via culture and PCR of various tissues, although results of the two methods did not always correlate. Pathology was generally related to the length of time the dis-

ease progressed, with all animals displaying varying degrees of severity of generalized disseminated TB. Acute multifocal necrosis was seen in tissues from the vervet, granulomas with multinucleate giant cells were present in rhesus tissues, and caseous granulomas with mature fibrous connective tissue capsules were apparent in the cynomolgus.

Intradermal tests using MOT were uniformly negative in all species 2 weeks PI; most were predictive between 4 and 12 weeks PI but then were uniformly false-negative for the remainder of the study. Interestingly, the intrapalpebral tests were often positive at the 24- or 48-hour reading but were consistently graded one to two grades lower (and therefore negative) at the definitive 72-hour reading. Intraabdominal testing was consistently less sensitive than intrapalpebral. Intradermal abdominal testing with PPD was routinely negative in all species throughout the study. Thoracic radiography was found to be predictive in all species. Radiographic evidence of pulmonary disease preceded most clinical signs, and lesion severity progressed with time. All vervets had radiographic evidence of pulmonary consolidation by 6 weeks PI and all rhesus by 3 months PI. Two cynomolgus monkeys had radiographic evidence of pulmonary disease by 1 month PI, two by 4 to 5 months PI, and two were radiographically unremarkable throughout the study period. No spondylitis or extrathoracic lesions were evident. Tracheal washes were rather predictive of infection as well. *M. tuberculosis* was identified by PCR or recovered by culture from tracheal washes of all animals, except for three cynomolgus, from at least one sampling point throughout the study. Frequent (in this case, every other week) sampling was necessary, however, as more individual samples were negative than positive.

Erythrocyte sedimentation rates (ESRs) were predictive in the vervets, with all five having prolonged rates at 1 month PI. ESR was not predictive in rhesus or cynomolgus. Hematology and clinical chemistry was not predictive of disease in any of the species. ELISA results were intriguing, but inconsistent. Three vervets seroconverted by 4 weeks PI and two did not. Five rhesus seroconverted by 6 weeks PI; one was not seropositive until 5 months PI. Cynomolgus seroconverted from 4 to12 weeks PI. Not all animals remained consistently seropositive after seroconversion, with five of the cynomolgus having biphasic or triphasic responses. Results of the lymphocyte proliferation assay also were somewhat interesting and tended to mirror the ELISA results. Coughing was the most predictive clinical sign noted in this study. Vervets displayed intermittent to chronic coughing from about 4 weeks PI until terminated; rhesus coughed from 1 to 3 months PI to around 5 months PI, and three of six cynomolgus coughed starting at 3 to 4 months PI continuing through 5 to 6 months PI.

In conclusion, we found that vervet monkeys are more sensitive to pulmonary *M. tuberculosis* than rhesus monkeys, which are more sensi-

tive than cynomolgus. We also found that it is important not to rely on intradermal tuberculin testing with MOT as the sole diagnostic method for tuberculosis because animals had many false-negative reactions. Furthermore, intraabdominal testing with PPD had no predictive value in this study. We found that thoracic radiography and assessment of coughing may have predictive value in diagnosing tuberculosis, as does culture or PCR testing of tracheal washes. The ELISA and lymphocyte proliferation assay may hold promise as adjunct diagnostic methods for tuberculosis.

Finally, it is clear that no single diagnostic test is absolutely predictive of TB in these Old World nonhuman primates. It is our recommendation instead to rely on a battery of tests to diagnose infection and to utilize a herd health approach to analyzing information instead of relying on individual animal data. It is clear that research is needed to discover and validate diagnostic methods that are sensitive, specific, and robust and utilize samples obtained in a minimally invasive manner.

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Specific Pathogen-free Rhesus Macaques

Keith Mansfield, DVM

INTRODUCTION

According to the World Health Organization, the human immunodeficiency virus (HIV) is recognized as the leading infectious cause of human morbidity and mortality worldwide. More than 30 million individuals have contracted this agent, with the vast majority of infections occurring in underdeveloped regions of the world. Development of effective strategies for control, prevention, and treatment remains a significant biomedical objective that must be addressed as we move into the next millennium. Rhesus macaques infected with simian immunodeficiency virus (SIV) represent one important tool with which to advance our knowledge of this disease, and these animals have been used extensively to investigate aspects of viral pathogenesis and host immunity. For reasons of biosafety and to eliminate confounding variables, such investigations are best carried out in animals that are free of a number of viral agents commonly found in conventional colonies, including simian retrovirus type D (SRV-D), simian T-lymphotropic virus (STLV-1), SIV, and B virus (CDC 1987; Holmes and others 1995). In addition to these requirements, investigators increasingly request animals of defined major histocompatibility complex (MHC) type or animals free of additional in-

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fectious agents in pursuit of their research goals. MHC-defined macaques have been utilized to investigate cytotoxic T-lymphocyte (CTL) responses during SIV infection through the use of tetramer technology. Animals free of additional agents have been utilized to investigate novel vaccine strategies and the pathogenesis of opportunistic infections during AIDS. Such requirements are likely to increase with further refinements of the model.

Animals specific pathogen free (SPF) of these agents are both costly and not readily available. The establishment of SPF colonies has proven difficult, and current production from domestic sources is not adequate to meet demand from private and academic institutions. The limited availability of such animals is an impediment to the continued progress and success of animal model-based AIDS-related research.

NEW ENGLAND NATIONAL PRIMATE RESEARCH CENTER SPF COLONY

A rhesus macaque breeding colony free of the specific pathogens B virus (BV), STLV-1, SRV-D, and SIV was established at the New England National Primate Research Center (NENPRC) in 1988. Stringent virological testing to identify SPF foundation animals in NENPRC breeding groups and among macaques purchased from other domestic sources has formed the basis of this successful colony. Virological testing has been performed on site with techniques originally developed and used extensively by investigators at the Center. Through careful management, the colony has grown to 550 adult breeding and juvenile animals and approximately 150 infant macaques less than one year of age. Juvenile animals are sold and assigned to investigators at 2 to 2 1/2 years of age, and this colony has produced 1220 offspring to date.

Virological assessment of the SPF colony is performed on site and is integrated with the colony preventative health care program. All colony animals are examined, TB tested, and bled once every 3 months. Serum obtained at this time is banked and evaluated by enzyme-linked immunosorbent assay (ELISA) for antibodies to B viruses, SRV-D, STLV-1, and SIV. Viral antigen and ELISA plates are produced on-site through techniques previously described, and herpes simplex is used in lieu of B virus antigen for biosafety reasons (Daniel and others 1988).

On-site testing is utilized both because it is cost effective and because it has reduced turnaround time compared with commercial laboratories. Positive and negative ELISA values are set using commercially available software (Dynatech Laboratories). All indeterminate or positive ELISA readings are sent for conformational testing to the Simian Retrovirus Laboratory (N. Lerche, Davis, CA) or to the National B Virus Reference

Laboratory (J. Hilliard, Atlanta, GA). Viral isolation, PCR, and immunohistochemistry are available on site and are used in the investigation of selected cases. The colony has remained negative to simian retroviruses since 1990. As a result of this success, annual testing for the simian retroviruses was initiated in 1999. A single break in BV SPF status occurred in 1996, and BV testing continues quarterly. The testing algorithm used for derivation of SPF macaques may be seen in Figure 1.

SPF DEFINITIONS

SPF macaques may be defined as offspring arising from breeding programs in which selected agents have been eliminated through an extensive test-and-remove strategy of founder animals. SPF founders may

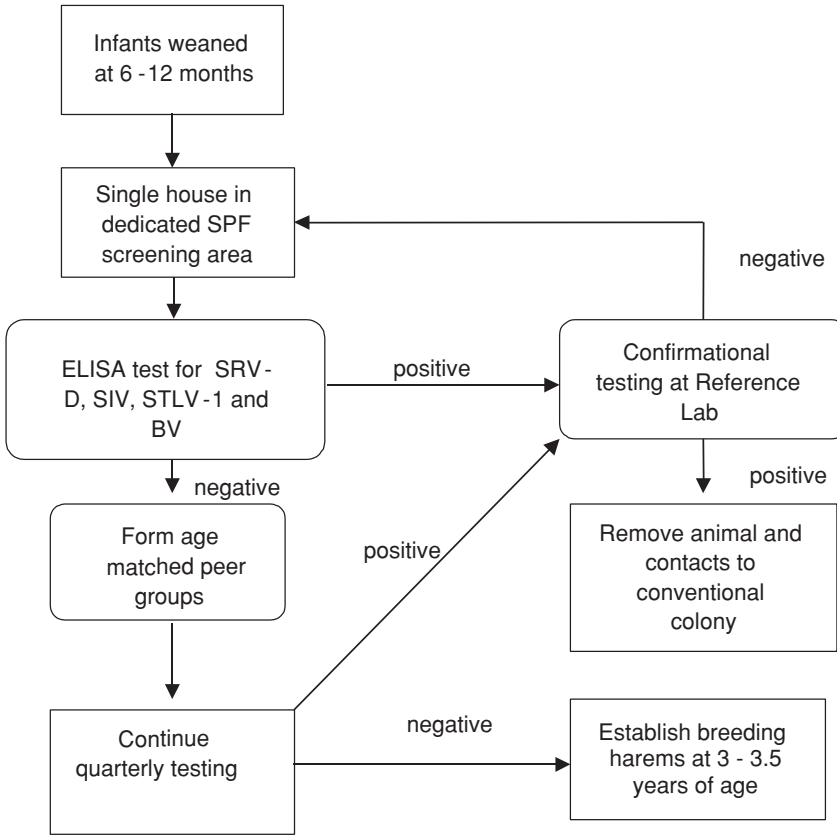

FIGURE 1 Testing algorithm for macaque SPF derivation.

also be known as level 1 SPF animals and offspring as level 2 SPF. By definition, SPF animals have been housed either individually or only with contact animals of similar virological status. "Conventional" animals are those in which no testing and segregation strategy has been employed. A "seronegative" designation is given to animals that arise from conventional colonies, lack antibodies to a particular agent, and have not been housed with other potentially infected animals. An animal lacking antibodies to a particular agent but housed with other infected animals should also be considered conventional.

SPF TARGET VIRUSES

The SPF target viruses may differ from colony to colony. The minimum target viruses for colonies supported by the National Institutes of Health/National Center for Research Resources are B viruses, STLV, SIV, and SRV-D.

SIV

SIVs are a group of related primate lentiviruses that have been investigated extensively as an animal model of human HIV infection (Gardner and others 1994). Numerous African primate species are naturally infected with specific strains of SIV with little clinical effect. Cross-species transmission from these species to Asian macaques results in a disease process remarkably similar to AIDS in humans (Baskin and others 1988; Lackner 1994). During the 1970s and 1980s, investigation of spontaneously occurring immunodeficiency in colony macaques housed at several National Primate Research Centers revealed that these animals had become infected with SIV (Lowenstine and others 1992; Mansfield and others 1995). Presumably, infection had resulted from cross-species transmission between captive animals. Although an antibody-negative viremic state exists, a simple test and removal strategy using standard whole virus ELISAs was successful in eliminating this virus from colonies (Daniel and others 1988). Whereas SIV is a target virus of most SPF colonies, spontaneous SIV infection of macaques has not been recognized in domestic colonies since the mid-1980s.

SRV-D

In spite of the extensive effort that has been devoted to the study of SIV, relatively little work has been conducted on SRV-D. The virus readily infects T cells (CD4 and CD8), B cells, macrophages, and epithelial cells (Lackner and others 1990). SRV-D is the principal cause of immunodefi-

ciency in domestically bred macaques and is a significant cause of morbidity and mortality when present in captive colonies. The natural host population for the SRV-D serotypes is unknown. SRV-D may be isolated from saliva of healthy carrier animals, and biting with inoculation of saliva or blood is the most likely method of horizontal transmission (Lerche and others 1986). Mother-to-infant transmission may occur in the perinatal and postnatal period. Clinical disease is characterized by chronic diarrhea and acquisition of opportunistic infections. Retroperitoneal fibromatosis and noma are clinical conditions associated with chronic SRV-D infection. As with SIV, a test and remove strategy has been successful in eliminating SRV-D (Daniel and others 1988). An antibody-negative viremic state occurs not uncommonly after in utero or perinatal transmission. Although this antibody-negative state may complicate testing strategies based on the detection of host immune responses, such animals generally present with clinical disease early in life, which can be eliminated by maintaining a high index of clinical suspicion and routinely attempting viral SRV-D isolation of ill animals. Alternatively molecular-based assays such as PCR or routine viral isolation can be incorporated in the initial screening program.

B VIRUS

B virus is an alphaherpes virus, which is similar to herpes simplex in humans and causes widespread infection of all species of macaques. B virus infection of macaques usually results in self-limiting clinical disease, with the establishment of latency after primary infection (Weigler and others 1993). The virus is readily transmitted, with most animals seroconverting by 2 years of age (Kessler and Hilliard 1990; Weigler and others 1990). Although consequences in the natural host are limited, zoonotic transmission of B virus to human handlers may result in severe clinical disease and often death. Even though it is a rare occurrence, with fewer than 40 fatal cases described in literature since 1932, many facilities will not accept B virus antibody-positive animals for reasons of biosafety (Holmes and others 1995). Of the four target viruses, B virus has been the most problematic to eliminate from SPF colonies (Weigler and others 1990), probably for multifactorial reasons. A phenomenon of delayed seroconversion has been recognized clinically in SPF macaque colonies since their inception. Delayed seroconversion can be defined as cases in which animals that have repeatedly tested negative for B virus for more than 1 year develop B virus antibodies (Freifeld and others 1995; Ward and Hilliard 1994). The phenomenon is poorly understood, but one hypothesis is that animals that were infected early in life may establish

latency without an adequate antibody response. These animals may then develop a primary humoral immune response during periods of viral reactivation. Regardless of their SPF status, it is common policy to treat all macaques as if they potentially harbor B virus and take appropriate precautions.

STLV

STLV is a type C retrovirus related to human T-lymphotropic virus type-1 (Franchini and Reitz 1994; Watanbe and others 1985). STLV appears to be less readily transmitted in macaque colonies with reported seroprevelance rates of 0 to 20%. Serological surveys indicate an increasing prevalence with age, and although the mechanism of natural transmission is unknown, parenteral and sexual routes are suspected of being of greater importance than perinatal transmission (Ishikawa and others 1987). Seronegative virus-positive animals are uncommon. STLV appears to have limited health consequences in immunologically normal macaques, but it has been associated with lymphoproliferative disorders such as lymphoma in AIDS (Homma and others 1984). STLV has limited zoonotic potential but has been selected as a target SPF virus due to its potential as a confounding variable in immunological studies, particularly those investigating other simian retroviruses. Commercially available ELISA reagents to detect antibodies to HTLV-1 also detect antibodies to STLV and can be used in a test and remove strategy (Daniel and others 1988). All positive tests should be verified by Western blot at a reference laboratory because the test used in this setting may lack specificity.

OTHER POTENTIAL SPF TARGET AGENTS

In addition to these four SPF target viruses, nonhuman primates may be infected with a variety of other viral bacterial and parasitic agents (Ward and Hilliard 1994). Such agents may adversely affect colony health or represent a significant zoonotic risk. Pathogen-free animals may also be required for specific research protocols. Examples of such agents are listed in Table 1. Many facilities have developed or are developing "expanded" SPF program in which animals are free of other agents in addition to the original four target viruses. Such expanded programs should be based on the present and anticipated research needs of facilities being supplied by the breeding program. In establishing new colonies, it may be educational to screen a subset of the source colony for additional agents. If the seroprevalence level of such additional agents is low or nonexistent, it may be cost effective to include these agents in the SPF program.

TABLE 1 Potential Additional Specific Pathogen-free Target Agents

Agent	Zoonotic Potential	Comment
Viral		
Rhesus rhadinovirus (RRV)	Unknown	RRV-free animals may be required for specific research protocol
Lymphocryptovirus (LCV)	Unknown	LCV-free animals may be required for specific research protocol
Rhesus cytomeglovirus (RhCMV)	Unknown	RhCMV-free animals may be required for specific research protocol
Simian foamy virus (SFV)	Yes	May confound studies; zoonotic potential
Simian virus 40 (SV40)	Yes	SV40-free animals may be required for specific research protocols; zoonotic potential
Bacterial		
Helicobacter pylori	Yes	*H. pylori*-free animals may be required for specific research protocols
Parasitic		
Cryptosporidia parvum	Yes	Potential zoonosis. *C. Parvum*-free animals may be required for specific research protocol
Enterocytozoon bieneusi	Yes	*E. Bieneusi*-free animals may be required for specific research protocols; zoonotic potential

VIRAL TESTING

A test-and-remove strategy based on detection of viral-specific antibodies is the foundation for establishing colonies free of target agents. Often screening and confirmational tests are utilized. The ideal screening test would be inexpensive, sensitive, specific, and technically simple to perform. Antibody detection-based ELISA procedures fulfill these criteria and have been used extensively in the establishment of SPF colonies.

On-site screening of samples by ELISA provides some advantages over sending all samples to reference laboratories for analysis. On-site testing is less expensive and has a shorter turnaround time. With proper planning, tests can be run within 24 to 48 hours of collection and can greatly expedite colony management decisions. Delays in obtaining re-

sults may be extensive, particularly if samples must be sent from the country of origin for testing. Such delays may adversely affect husbandry decisions.

ELISA plates may be made for the four target viruses utilizing purified whole virus preparations (Daniel and others 1988). HSV-1 may be used in lieu of B virus for biosafety reasons. HSV-1 and HTLV-1 plates are also available commercially and can be adapted for use in a surrogate testing program for B virus and STLV-1, respectively. SRV-D and SIV plates are not currently available commercially and must be prepared from purified virus. A summary of viral preparation and ELISA techniques currently in use at the NENPRC is presented in Table 2.

Antibody testing for B virus may lack sensitivity in certain instances. Seroconversion of SPF founder animals has previously been described and represents a problem in the establishment of SPF colonies. It is suspected that delayed antibody response plays a role in the virus-positive result of tested animals (Ward and Hilliard 1994). Whether such animals shed virus and remain a threat to other colony animals and humans remains unknown. In 1996, it was noted that indeterminate values utilize the HSV ELISA in a NENPRC female SPF founder. Previously this animal had been negative on repeated HSV ELISAs performed at NENPRC and on a Western blot analysis performed at the National B Virus Reference Laboratory in 1992. Following recognition of the indeterminate ELISA, confirmational testing was requested from the National B Virus Reference Laboratory that confirmed B virus antibody by Western blot. This animal

TABLE 2 Parameters in Specific Pathogen-free Testing at NENPRC[a]

Viral purification
- Cells for virus production grown in 600-mL T-150 flasks
- Harvested by centrifugation 72 hr after splitting
- Cell-free supernatants pelleted by ultracentrifugation (48,000 *g* at 4°C for 3 hr)
- Purified by sepharose 4B column chromatography
- ELISA[a] plates coated with viral antigen diluted 1:1,000 in Triton ×-100-PBS[a] and blocked with 0.3% BSA[a]

ELISA
- 1:20 dilution of test serum (1hr)
- 1:100 dilution of goat anti-human immunoglobulin G (Fc) conjugated with AP[a] (1 hr) (Kirkegaard & Perry Laboratories)
- Developed with *p*-nitrophenylphosphate substrate (30 min)
- Absorbance read at 410 nm with Dynatech ELISA reader

[a]AP, alkaline phosphatase; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; NENPRC, New England National Primate Research Center; PBS, phosphate-buffered saline.

and contacts within the breeding harem were removed from the colony. Further testing did not reveal seroconversion of additional animals in the group, and the colony has remained negative to B virus based on continued quarterly testing. This case illustrates the problem experienced in attempting to eradicate B virus from macaque colonies. Although of concern, this is the only such case recognized at the NENPRC colony since 1992, and it represents more than 3000 macaque-years of observation. As in other institutions, the NENPRC maintains a policy that all macaques should be treated as if they harbor B virus and appropriate precautions taken.

TESTING STRATEGIES

Testing strategies may vary depending on the founding population and target SPF agents, and they should be tailored to the individual facility. A testing strategy is outlined in Figure 1. In general, juvenile animals between 6 and 12 months of age are the best SPF candidate animals. Maternal immunity offers a level of protection, and most animals do not seroconvert to the target viruses until 2 to 3 years of age. Although animals may be used at a younger age, this approach increases husbandry requirements and may result in increased health problems and behavioral disorders. Healthy juvenile animals are weaned from founder breeding groups at 6 to 12 months of age. At weaning a blood sample is obtained and routine preventative health care performed. Animals are housed individually until initial virological testing is available. Seropositive animals are returned to the conventional colony. Seronegative animals are placed in small age-matched peer groups of 2 to 4 animals. Small peer groups allow for normal behavioral development but limit contact to prevent spread of infectious agents. In general, a 1:8 male to female ratio is used in the harvest. Animals are examined quarterly, and blood is drawn for serological assessment. Indeterminate or positive ELISA tests obtained at this stage should be confirmed at a reference laboratory. If seroconversion is detected, the entire group should be removed to the conventional colony. Quarterly testing should continue for a minimum of 2 years, and animals should be placed in breeding harems at 3 to 3 1/2 years of age. Due to difficulties experienced in eliminating BV, quarterly testing for this agent should continue indefinitely.

HOUSING STRATEGIES

Housing strategy is an important component of SPF colony management and may directly affect program success. A variety of strategies have been used to house SPF breeding colonies including time mating,

corral breeding, and harem breeding configurations. All strategies offer distinct advantages and disadvantages (summarized in Table 3). Corral housing is a common configuration used in areas of the world in which year-round climatic conditions allow housing of animals outdoors. Corrals generally contain 15 to 20 breeding males and 100 to 125 adult females. Corral configurations have reduced fixed and variable costs including reduced construction, labor, and supply costs. Reproductive efficiency is usually greater than with other housing configurations. Corral housing presents several distinct disadvantages from the standpoint of establishing SPF colonies. The main disadvantage is that pathogen containment is more difficult in large group settings. A single break in SPF status puts all contact animals at risk. When this occurs in breeding harems, potential contacts have been limited to the 8 to 10 animals housed together. Testing of the affected group can be intensified or the entire group can be eliminated. A break in SPF status in corral housing may put 150 to 200 animals at risk, and a subsequent test-and-remove strategy is difficult or impossible to perform.

In contrast, breeding harems consist of one male and 8 to 10 adult females. Animals are housed in indoor pens or out-door "corn cribs." This smaller size effectively contains breaks in SPF status. Breeding harems also allow for the more accurate construction of breeding pedigrees and enhance the ability to observe sick or injured animals. Smaller group size increases the ability to observe and treat injured animals and to perform routine preventative health care.

Time mating strategies offer similar advantages to breeding harems in pathogen containment but have high caging costs, are labor intensive, and often have reduced reproductive performance. They allow the production of timed pregnancies for research purposes.

TABLE 3 Advantages and Disadvantages of Breeding Strategies in Production of Specific Pathogen-free Macaques

Breeding Corrals	Breeding Harems
Increased reproductive efficiency	Decreased reproductive efficiency
Decreased initial construction costs	Increased initial construction costs
Decreased husbandry costs	Increased husbandry costs
Difficult to pedigree	Reliable pedigrees
Increased overall mortality	Decreased overall mortality
Difficult pathogen containment	Ease of pathogen containment

PREVENTIVE HEALTH PROGRAM AND DISEASE SURVEILLANCE

A strong preventative health care program is critical to the success of SPF colonies. Such programs allow not only the tracking of target SPF agents but also the control of other potential primate pathogens that affect colony health. Components of a preventative program should include quarterly routine physical examination of all animals. At that time, intradermal tuberculin testing and phlebotomy for viral testing can be performed. Serum and, optimally, DNA should be obtained and stored. Vaccinations based on risk assessment for measles, rabies, and tetanus may be administered. Standard operating procedures for these procedures should be developed and followed. Individual animal medical records should be maintained.

A rigorous diagnostic program for ill or sick animals should be developed to help in the identification of potential disease outbreaks that may affect colony health. A production-animal herd-health approach should govern the decision making process. Disease surveillance is an important component of a preventative health program. All animals that have died or are euthanized for poor health should be necropsied, and tissue should be examined histologically. Results should be entered in a computerized database to allow tracking of disease trends. Necropsies allow not only identification of cause of death in individual animals but also surveillance of the colony and identification of subclinical disease.

Strict separation of SPF colony animals from conventional colony animals, indigenous primates, and other wild or feral populations must also be maintained. Indigenous primates may represent a potential exogenous source of the SPF target agents. Wild or feral populations such as rodents and dogs may transmit such important pathogens as leptospirosis, encephalomyocarditis virus, or rabies virus. Once colonies are established, introduction of animals from other sources should be discouraged. If required to maintain genetic diversity, extended quarantines and repeated testing for a period of at least 1 year is recommended.

EXPANDED SPF PROGRAMS

When the NENPRC SPF colony was first established, four viruses were targeted for elimination: B virus, STLV-1, SRV-D, and SIV. Since then, additional viral agents have been recognized as causing widespread infection in most rhesus macaque colonies. With refinement of the SIV AIDS model, investigators have requested animals free of additional agents to study the impact of concurrent infections on disease pathogenesis and to investigate novel antigen delivery systems. Particular colonies

free of SV40 and SFV may be required for some experimental protocols and would represent a valuable SPF asset. Furthermore, in some cases (e.g., simian foamy viruses), these agents may pose a newly recognized zoonotic risk to personnel. In response to such requests, the NENPRC has rederived animals from the SPF colony by cesarean section. These animals were hand reared in isolation from the remainder of the colony and tested for the presence of additional viral and nonviral pathogens. Quarterly testing by ELISA or PCR has revealed that animals are free of the following agents:

- Simian virus 40 (SV40), based on ELISA;
- Lymphocryptovirus (LCV), based on PCR/peptide ELISA
- Rhesus rhadinovirus (RRV), based on ELISA
- Cytomegalovirus (CMV), based on ELISA and PCR
- Simian foamy virus (SFV), based on ELISA

To perform this testing, additional ELISA procedures were developed using purified virus for SV40, RRV, and CMV as previously described (Desrosiers and others 1997; Kaur and others 1996). Testing for LCV was initially performed by PCR on DNA isolated from peripheral blood mononuclear cells as previously described. A peptide ELISA for antibody testing has also been developed utilizing two peptides representing the carboxyterminal domains of the rhesus LCV VCAp18 (Wang 2001).

SUMMARY AND RECOMMENDATIONS

- **Consider minimum SPF target viruses: B virus, SIV, STLV, and SRV-D.** Consideration should also be given to the inclusion of other agents such as RRV, CMV, LCV, SV40, and SFV based on perceived research requirements and seroprevalence in the source colony.
- **Select on-site serological testing capabilities/off-site confirmational testing.** For cost effectiveness and rapid responsiveness on-site serologic testing capabilities may be warranted. Colonies should have access to reference laboratories for off-site confirmational testing.
- **Perform initial serological surveys.** Serological surveys should be performed during the SPF planning process to assess the prevalence of the target agents and to define other agents that may or may not be present in the colony.
- **Select candidate SPF animals at 6 to 12 months of age.** Most macaques will seroconvert to the four standard target SPF viruses between 1 and 3 years of age. Initial testing of animals at 6 to 12 months will increase the seronegative selection pool.

• **House animals initially in small peer groups and test quarterly for target viruses for 2 years.** After initial tests, seronegative animals should be formed into small peer groups of 2 to 4 animals, and testing should continue quarterly until negative for 2 years. Animals that seroconvert and contacts should be removed from the colony.

• **Establish breeding harems at 3 to 3 1/2 years of age and begin testing for retroviruses annually and BV quarterly.** There are advantages and disadvantages to the various possible SPF breeding configurations. Breeding harems provide the best opportunity at pathogen containment early in SPF colony development.

• **Separate SPF colonies from conventional and indigenous primates.** Strict separation of SPF colonies from conventional and indigenous primates as well as feral and wild animal populations should be maintained. Such primates represent a potential source of breaks in SPF status.

• **Establish a vigorous preventative health care and diagnostic program.** This program should include quarterly physical examinations, blood and DNA banking, viral screens, vaccinations, and individual animal medical records.

• **Maintain genetic diversity.** SPF animals should be managed to preserve genetic diversity and maintained as out-bred colonies. Detailed pedigrees and breeding records should be kept and the use of software for the management of genetic, pedigree and demographic data considered.

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Session 5: Panel Discussion

Participants:
Hilton Klein—Session Chair, Merck Research Laboratories, USA
Gary B. Baskin—Tulane National Primate Research Center, USA
Tom DeMarcus—CDC/National Center for Infectious Diseases, USA
Sherri L. Motzel—Merck Research Laboratories, USA
Keith Mansfield—New England National Primate Research Center, USA

QUESTIONS AND ANSWERS

PARTICIPANT A: Dr. Motzel, did you see anything interesting on the bronchial lymph nodes on those monkeys at post mortem examination and were they routinely stain-free acid-fast bacteria? If so, what did you see?

DR. MOTZEL (Sherri L. Motzel, Merck Research Laboratories): They were routinely stained for acid-fast bacteria. We also did polymerase chain reaction (PCR), and we had variable results. We saw acid-fast organisms in some cases, and we did see positive PCR on some. I think that of the tissues that were most predictive for recovering tuberculosis, they were probably in the top three along with lung and, in our case, the liver.

PARTICIPANT B: Mr. DeMarcus, do you know which species were the 18 DRAs on your list?

MR. DEMARCUS (National Center for Infectious Disease): I do not recall, but I can get that information for you. They were macaques.

PARTCIPANT B: Thank you. Do you perhaps know which origin or which country?

MR. DEMARCUS: I am sorry, I do not have that information with me.

DR. BAUDOIN (Mario Baudoin, Ministry of Sustainable Development and Planning): Dr. Mansfield, could you tell me the age of the female animal that was ADA negative until 1992, and then in 1995 was separated from the source colony.

DR. MANSFIELD (Keith Mansfield, New England National Primate Research Center): She was an adult animal when she was recruited to the colony, not a juvenile.

DR. BAUDOIN: Is this then probably the longest incubation time we have ever heard of herpes B?

DR. MANSFIELD: It is fairly long, but I think there are other cases of 6, 7, or 8 years after separation from the source colony.

I would like to add a quick comment: someone asked whether we treat our SPF for superclean animals any differently from the conventional animals, and we do not. We treat all animals as if they potentially harbor B virus and wear the same PPE. Part of that approach is to prevent the acquisition of B virus from an animal such as this. The other aspect is that these animals do carry a wide variety of other infectious agents that are potentially harmful to humans.

DR. BAUDOIN: Just out of curiosity, is it true that none of the other animals were in close proximity in your harems?

DR. MANSFIELD: None of the animals share a converted status; and as far as I am aware, no one has demonstrated that these animals that showed delayed seroconversion, if that is what it is, pose a risk to other animals in the colony—that they, in fact, shed virus.

DR. BAUDOIN: An additional matter of curiosity to me is whether we are certain this is herpes B and not something that cross-reacts with herpes?

DR. MANSFIELD: The sample was sent off to the National B-Virus Laboratory, and they distinguished HSB from herpes B. As reliable as that can be, it appears that whatever it was was closely related to herpes B.

PARTICIPANT C: Mr. DeMarcus, the importation information was very interesting, and I noted that the peak importation in the United States, 1988-1989, corresponded with the Ebola outbreak in Reston. Other than testing for the viruses, was there anything different about the quarantine procedures in 1988 versus what you are doing today?

MR. DEMARCUS: Certainly, most of the monitoring and surveillance that CDC was doing of primate quarantined facilities prior to the

Reston Ebola outbreak in 1989 and 1990 was passive. We maintained a registry. We required those registrations to be renewed every 2 years. We reviewed the information that was submitted in the registration applications. We routinely visited facilities, monitored their handling procedures, and reviewed their records. Beyond making additional recommendations for disease control, we required importers to document their compliance with those recommendations for continued registration. We monitored the handling of the animals on arrival at the airport, on arrival at the facility, and periodically; and we reviewed their compliance with the implementation of the standard operating procedures. So yes, it was dramatically different before and after.

PARTICIPANT C: As a follow-up question, has any one agency historically looked at the time period to try to trace back the origin of the infection in the Reston animals to where they might have been contaminated or whether they were contaminated at the source of origin?

MR. DEMARCUS: Yes, I think there are several papers in the literature, and there is also some special pathogen personnel still present at the CDC who could probably answer that question better than I can.

PARTICIPANT C: Dr. Mansfield, when you do SRV testing and on Western blot, your confirmation test, what do you do when you obtain an indeterminate, particularly on epitose 27s?

DR. MANSFIELD: We do all our confirmational testing at the National Retrovirus Laboratory, and we do have occasional animals that are in the SPF colony that show indeterminate tests on Western blots. When we follow those animals, often they revert to a negative test over a period of time. I believe those animals are negative because none of the animals' contacts, have ever seroconverted to D virus in the past 12 years.

PARTICIPANT D: First, Mr. DeMarcus, I was somewhat surprised that the CDC does preshipment diagnostic testing given the many properties these monkeys have and the fact that the animal handlers at the airport may be exposed to these conditions. I am wondering whether the CDC revealed these requirements to actually make it compulsory for some sort of preshipment testing to try and minimize that danger of infection at the airport or for the animal handlers.

MR. DEMARCUS: I think that the question of preshipment testing is a complicated one. It would require standardization, oversight, and monitoring, which the CDC really does not have the resources to implement. We believe that if the recommended precautions regarding separation and isolation during transit and quarantine are implemented properly, that the risk during transit is very low and the screening done in quarantine is more reliable to make certain that the animals are at least free of disease before they are released from quarantine.

PARTICIPANT D: Dr. Motzel, with regard to the animals that were experimentally infected with *Mycobacteria*, did you attempt to treat them and if so, was the treatment regime successful?

DR. MOTZEL: We did not treat any of the animals.

DR. MCGREAL (Shirley McGreal, International Primate Protection League): Mr. DeMarcus, I am thinking of the reports in 1989 when they had facilities with 49 (between 13 and 49) deficiencies of monkeys in two-tier cages urinating on top of those below, which was totally appalling. Do you think these kinds of conditions have stopped, and secondly, how can one justify allowing macaques into the pet trade? Anybody can answer this question because I think the CDC is probably the one agency that can stop it rather than trying to obtain restrictive state-by-state laws, because someone with a pet macaque will die, which we want to prevent.

MR. DEMARCUS: The conditions in primate quarantine facilities comply with the CDC recommendations that were implemented in 1989 and 1990 without exception. As I mentioned in my presentation with respect to specific disease, CDC's regulatory mandate is aimed at those diseases that have the capability of causing serious outbreaks—person-to-person spread in humans. Although B virus is a potentially deadly disease in humans, it is very rare and rare indeed in its reported ability to be able to spread person-to-person. Again, CDC's mandate is to prevent serious outbreak of human disease associated with nonhuman primates. CDC believes that the 31-day quarantine is adequate to do that. Certainly macaques do not make good pets. They present serious hazards to advocationists, but it is beyond the scope of CDC's import quarantine mandate to attempt to regulate the possession and interstate distribution of nonhuman primates.

PARTICIPANT E: Dr. Mansfield, is anyone developing SPF colonies of Chinese rhesus?

DR. MANSFIELD: Not that I am aware of. All the OAR funded-breeding programs are Indian-origin rhesus macaques.

PARTICIPANT E: Do you think it would be worthwhile to do so?

DR. MANSFIELD: Yes, I think it would be worthwhile.

DR. ROBERTS (Jeffrey A. Roberts, University of California—Davis): I can respond to that question because we have a base grant SPF colony and a colony supported through the grant mechanism, and our base grant colony does include Chinese SPF animals in our stock. I have two questions, one very specific and one more general. Dr. Mansfield, in terms of the rabies vaccine, what was the product and what was the rationale for indoor-housed animals?

DR. MANSFIELD: To clarify, I am saying that the vaccination program needs to be based on risk assessment. So for an indoor-housed

animal in New England, rabies is relatively low on my differential list. However, certainly in other places in the world, rabies may be much more problematic. Then I think there should be a risk assessment study performed to determine whether that vaccine might be worthwhile to use. A variety of vaccines that have been used in nonhuman primates; however, the efficacy is unknown. I do not think anyone has done studies to show that they are efficacious.

DR. ROBERTS: The second question is for all of the pathologists on the panel. In this session, we have talked about diseases and about the importance of record keeping and allocation of resources. What do you do in terms of banking tissue samples; how long do you keep them? It is a tremendous information database, but it also takes up a lot of space and requires a lot of inventory. Is there any feedback you would like to give on your policies?

DR. BASKIN (Gary Baskin, Tulane National Primate Research Center): We keep blots and slides forever. We keep wet tissues until the case is signed out. On routine animals, animals that are on specific projects, we ask the investigator whether to store the wet tissues after the pathology work is complete. Our problem is storage capacity. We perform approximately 900 micropsies per year. You can imagine the storage problems with those kinds of samples, and we do not keep them.

DR. MANSFIELD: We have a smaller colony and probably do about 300 to 350 micropsies per year. We keep all paraffin-embedded, wet, and frozen tissue, at least as far back as 1988. All animals' micropsy have both formal and fixed and frozen tissue on them, but it is becoming a space issue for us also.

DR. MOTZEL: We keep all blots and slides, but we routinely dispose of wet tissues after a period of a few years.

PARTICPANT F: I have been working with John Robertson at the Regional College of Veterinary Medicine at Virginia Tech to begin developing a tissue archive for primates. Hopefully it would be possible to develop a repository there, in a relatively low cost area, where additional slides, tissues, and specimens could be stored for any of you. Those of you who have just mentioned some of the problems with repositories eventually will probably be filled. This project is pursuant to our development of a great ape brain bank, which is now being expanded into a CNS tissue repository beyond great apes. I mention it because it has already been very productive, particularly in alliance with some of the human brain banks that are supported under the Alzheimer's program.

Session 6

Transportation

OLAW Perspective on Transportation of Nonhuman Primates

Nelson Garnett, DVM

I The US Fish and Wildlife Service issues wildlife-related permits.
II The National Center for Infectious Diseases, Division of Global Migration and Quarantine also has regulations governing the importation of pets and other animals into the United States.
III The USDA Animal and Plant Health Inspection Service, National Center for Import and Export.
IV Jurisdiction
  A Public Health Service Policy on Humane Care and Use of Laboratory Animals.
  B Guide for the Care and Use of Laboratory Animals
    1 Guide Standards
      a Minimize transit time
      b Minimize risk of zoonoses
      c Protect against environmental extremes
      d Avoid overcrowding
      e Provide food and water
      f Protect against physical trauma
V Transportation
  A "In most cases in the United States, nonhuman primates are transported in motor vehicles with self-contained climate control units

Office for Laboratory Animal Welfare, NIH, Bethesda, MD

separate from the driver's compartment, or by commercial airliners. Before shipping animals, one should carefully review all applicable laws and regulations. The following points are relevant to the transportation of nonhuman primates: …"

B "Transportation of nonhuman primates requires adherence to the standards published in the Code of Federal Regulations as well as those pertaining to interstate/international movement of animals if applicable. In addition, see Chapter 3 for information to comply with the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the United States Endangered Species Act (ESA)."

C "The International Air Transport Association (IATA) publishes requirements for international shipping. These include detailed specifications for shipping crates, isolation of animal shipments from other cargo, handling of loaded crates for personnel safety, and many additional requirements covering a multitude of issues…."

D "Airlines are not required to carry animal shipments, and many airlines have opted not to handle these shipments. Consequently, there are fewer options for shipping these animals."

VI Transportation Initiative

A Regulatory burden item

B Offshoot of OLAW meetings on field biology and international collaboration

C Informal inquiry from IACUC Chair to OLAW, AAALAC, USDA, AALAS representatives regarding transportation problems

D Identified need for ILAR study on research animal transportation issues

1 Not limited to nonhuman primates

2 Increased complexity due to homeland security concerns

a Improved quality/welfare

b Availability of services

c Reduction of burden

d Transport of tissues/specimens

e Biosecurity concerns

3 NIH/OLAW contribution

4 Partners needed

Transportation of Primates and the Animal Welfare Act

Jerry DePoyster, DVM

The Animal Welfare Act (AWA) was first passed in 1966. A provision providing for the humane transport of "animals" was not added until a 1976 amendment. In the AWA, a nonhuman primate is defined as an "animal" for purposes of the AWA when it is being used, or intended for use, for research, teaching, testing, experimentation, or exhibition purposes, or the breeding of, or the selling of, as a pet. Ownership of nonhuman primates used solely as pets is not under the jurisdiction of the AWA.

AWA transportation standards are detailed in 9 CFR, Part 3, Section 3.86 through 3.92, and in 9 CFR, Part 2, Section 2.131. The titles of these particular standards and a bulleted summary of the contents are listed below.

9 CFR, Part 3

Section 3.86: Consignment to carriers and intermediate handlers

- Record keeping
- Time restrictions
- Environmental factors, acclimation certificate
- Feed and water instructions

USDA, Animal and Plant Inspection Services, Riverdale, MD

Section 3.87: Primary enclosures used to transport nonhuman primates
- Construction
- Cleaning
- Ventilation
- Compatibility of animals in enclosure
- Space requirements
- Marking and labeling
- Record keeping

Section 3.88: Primary conveyances (motor vehicle, rail, air, marine)
- Cargo space design
- Placement of primary enclosure
- Environmental factors

Section 3.89: Food and water requirements
- Time restrictions
- Placement of receptacles

Section 3.90: Care in Transit
- Observation of animals and environment
- Veterinary care if needed

Section 3.91: Terminal facilities
- Placement of primary enclosure
- Cleaning, sanitization, and pest control
- Ventilation
- Temperature
- Shelter
- Duration of stay in terminal facility

Section 3.92: Handling
- Environmental factors and shelter
- Care of container while handling

9 CFR, Part 2
Section 2.131:Handling of animals

(a)(1) Handling of all animals should be done as expeditiously and carefully as possible in a manner that does not cause trauma, overheating, excessively cooling, behavioral stress, physical harm, or unnecessary discomfort.

The full text of these standards, which appears in the document, is beyond the scope of this volume.

Many of the major worldwide air carriers are members of the International Air Transport Association (IATA) and comply with IATA's transportation regulations. The IATA and AWA regulations have very similar requirements with one major difference—the IATA regulations do not

allow transportation of pregnant females and females with suckling young whereas the AWA does allow a mother and nursing infant to travel together. There are also differences in the temperature ranges during different stages of transportation. The AWA allows from 45°F to 85°F whereas the IATA has an optimum temperature range from 70°F to 90°F. For all intent and purposes (and although some differences may have been overlooked in this very brief review), if IATA guidelines are being met, then it is most likely that the AWA regulations are also being met except for differences such as those stated above. It must be said that because the AWA is a law and the IATA regulations are member guidelines, transporters in the United States must fulfill the mandates of the AWA. Therefore, even though they may be an IATA member and meeting IATA regulations, transporters should also review the AWA standards before shipping to, from, or from one point to another in the United States. Outside the United States and its territories, the AWA does not apply.

Other than the US Department of Agriculture's (USDA's) and IATA's standards, there are other requirements that the transporter should be aware of. For example, only those nonhuman primates to be used for educational or scientific purposes are allowed entry into the United States (Centers for Disease Control and Prevention [CDC]). In addition, a list of these requirements would include, but is not limited to, the following:

- Health Certificates (USDA and individual state government requirements);
- Permit from the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) (US Fish and Wildlife Service);
- Quarantine and tuberculosis testing (CDC); and
- Restrictions on wood from certain countries because of nonnative pests (USDA).

For various reasons, air carriers may be reluctant to carry nonhuman primates. USDA's jurisdiction covers only the humane care of animals that are transported and not whether or not air carriers transport animals. We believe, and this is only conjecture, that the air carriers could be reluctant to carry nonhuman primates for reasons that may include one or more of the opinions stated below:

- Health issue concerns for nonhuman primates and humans are prevalent.
- Animal cargo handlers must be trained and outfitted to wear full biohazard gear. The gear is also expensive.
- Insurance rates may be higher due to health and liability concerns.

- Airline passengers have a negative response to seeing handlers in full biosuits unloading cargo. It is possible that animal handlers may also have concerns about wearing this gear not only because it is awkward, but also for their health and well being.
- Cargo areas must be disinfected after each shipment of nonhuman primates.
- It is not uncommon for escapes to occur during transportation, thus endangering the animal and the public.
- Pressure from animal welfare/rights organizations.

Although USDA/Animal Care presently regulates international transportation, plans are to begin full regulation of all international carriers of animals while on United States soil. Listed below is contact information for USDA's Animal Care offices.

Headquarters
USDA-APHIS-Animal Care
4700 River Road, Unit 84
Riverdale, MD 20737-1234
Phone: (301) 734-7833
Fax: (301) 734-4978
Email: ace@aphis.usda.gov

Western Region
USDA-APHIS-Animal Care
2150 Centre Ave., Building B
Mailstop # 3W11
Ft Collins, CO 80526-8117
Phone: (970) 494-7478
Fax: (970) 494-7478
E-mail: acwest@aphis.usda.gov

Eastern Region
USDA-APHIS-Animal Care
920 Main Campus Drive, Suite 200
Raleigh, NC 27606-5210
Phone: (919) 716-5532
Fax: (919) 716-5696
E-mail: aceast@aphis.usda.gov

International Transportation of Nonhuman Primates: US Fish and Wildlife Service Perspective

Michael Kreger, MS, PhD, and Monica Farris†*

There are many regulatory issues concerning the care and use of nonhuman primates in biomedical research in the United States. Just as the Animal Welfare Act, administered by the US Department of Agriculture, covers interstate transportation of live nonhuman primates, and the US Public Health Service registers importers and requires quarantine of nonhuman primates under the Public Health Service Act, laws administered by the US Fish and Wildlife Service cover the import and export of these species. Two laws affect the transportation of nonhuman primates to and from the United States: the Lacey Act and the Endangered Species Act of 1973 (ESA), which implements the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). In this presentation, we will provide an overview of how these laws regulate the transport of nonhuman primates.

LACEY ACT [18 USC 42]

The Lacey Act prohibits import, export, transport, possession, sale, and purchase of mammals and birds in violation of state, federal, and foreign laws or regulations. Enacted in 1900, the Lacey Act is the oldest

*Division of Scientific Authority
†Division of Management Authority, US Department of the Interior, Arlington, VA

federal wildlife protection law in the United States. Regarding humane transportation, it states,

> It shall be unlawful for any person, including the importer, to knowingly cause or permit any wild animal or bird to be transported to the United States, or any territory thereof, under inhumane or unhealthful conditions" (18 USC 42 (C)).
>
> The presence in such vessel or conveyance at such time of a substantial ratio of dead, crippled, diseased or starving wild animals will be deemed prima facie evidence of the violation of the provisions of this subsection" (18 USC 42 (C) (2)).

The regulations for transport under the Lacey Act (50 CFR Part 14, Subpart J, Humane and Healthful Transport of Live Mammals and Birds) closely resemble the guidelines found in the International Air Transport Association's *Live Animals Regulations* (LAR). In fact, the Lacey Act regulations are periodically amended to reflect the most current version of the LAR. The Lacey Act also applies LAR to nonairline methods of transport.

General rules for birds and mammals (§14.101-112) include the following:

- Carriers must have designated animal holding rooms/areas, free from exposure to noise, harassment, fumes, and away from inanimate cargo.
- Enclosures must have spacer bars.
- Enclosures must have upright arrows.
- Food/water/care instructions must be affixed to the outside of the container.
- Enclosures must have emergency access to animals.
- Enclosures must have sufficient openings to ensure adequate circulation of air at all times.
- The interior of the enclosure must be free from any protrusion that could be injurious to the animal.
- Animals must be provided safe, nontoxic litter in sufficient quantity to absorb and cover excreta.
- Animals must be provided food and water.
- Auxiliary ventilation must be provided when the surrounding air temperature exceeds 75ºF.
- An unweaned, nursing (with young), sick, or injured animal will not be transported to the United States.

Specifications for nonhuman primates (§14.121-123) include:

- No more than one primate per enclosure except under certain circumstances.

- Enclosures must be large enough for the animal to turn around freely, lie down, stand up, and sit in a normal upright position.
- Food and water must be provided at least once every 12 hours, unless otherwise instructed.
- A primate shall be observed for signs of distress and given food and water according to the shipper's instructions during any intermediate stop that lasts more than four hours.

ENDANGERED SPECIES ACT OF 1973 (ESA) [16 USC 1538 (C)]

The regulations for the ESA are found in 50 CFR Part 17. The ESA was passed to prevent the extinction of native and foreign animals and plants by providing measures to help alleviate the loss of species and their habitats. The objective is to rebuild wild populations of species in danger of extinction (endangered) or threatened (likely to become endangered in the near future without adequate monitoring and protection). All mammals and birds listed under the ESA in transport must comply with the provisions of the Lacey Act. In addition, the ESA is the law that implements the international CITES treaty (50 CFR Part 23).

The CITES treaty is intended to prevent the exploitation of wild animals and plants through international trade. Although some nonhuman primate species are listed as endangered or threatened under the ESA, all nonhuman primates are listed under CITES. The treaty relies on a basic principle of strictly limiting international trade in species in genuine need of protection while allowing controlled trade in species that are capable of sustaining some level of exploitation. A total of 157 nations are now Parties to CITES, with a few new countries still joining every year. CITES Resolution 10.21 endorses the LAR for both air transport and overland transport.

PERMITS FOR IMPORTS AND EXPORTS OF NONHUMAN PRIMATES

All nonhuman primate imports and exports require permits. CITES establishes a permit system for regulating international trade, including imports, exports, and re-exports, in certain taxa of plants and animals, and parts and products derived from them, whether live or dead. (It is important to understand that in a CITES context, "trade" comprises any movement of specimens across international borders, for any purpose, whether or not it is commercial.) The taxa covered by the Convention are listed in three appendices, which determine how the Parties apply import and export controls on specimens of the listed species. The level of listing also determines the types of findings that must be made for the issuance

of permits. A listing may cover an entire genus (e.g., all *Pan* species) or any lower taxonomic level. Currently, all nonhuman primates are listed in either Appendix I or Appendix II of CITES.

The most restrictive list is Appendix I, which includes taxa (e.g., apes, lemurs, many tamarins) threatened with extinction and for which trade must be subject to particularly strict regulation and only authorized in exceptional circumstances. Commercial trade in Appendix-I taxa is prohibited.

International transactions in Appendix-I taxa require both an import permit and an export permit. The treaty specifies that an import permit should be granted by the importing nation before the export permit is issued. For an import permit to be granted, the importing country must determine that (1) the import is not for primarily commercial purposes; (2) the import will be for purposes that are not detrimental to the survival of the listed taxon; and (3) if the specimens are alive, the recipient is suitably equipped to house and care for them. For the issuance of an export permit, the exporting country must conclude that (1) the specimens were legally acquired; (2) the export will not be detrimental to the species' survival; and (3) for live specimens, they will not be harmed during shipment. In practice, these provisions mean that legal trade in Appendix-I specimens is very restricted and that an import must be for a noncommercial purpose, such as scientific research, education, or conservation.

Appendix II includes taxa that, although not necessarily now threatened with extinction, may become so unless trade in them is subject to strict regulation to avoid utilization incompatible with their survival. Listing in Appendix II provides a mechanism whereby trade in exploited species can be monitored, thus providing information needed to regulate the trade so that it is sustainable. Each CITES Party has the responsibility to determine whether its traded species are regenerating in nature at a sufficient rate to be self-sustaining.

The conditions for trade in specimens of Appendix-II species are less strict than those for Appendix-I species, since no CITES import permit is required (although a country may have other laws requiring that import permits be issued). The exporting country must make the same determinations for export of specimens of an Appendix-II species as for those of an Appendix-I species (i.e., that the specimens in question were legally obtained, and the export will not be detrimental to the survival of that species). The exporting nation must also be satisfied that any living specimen will not be harmed during shipment, and they must monitor trade to ensure that Appendix-II species are maintained at an ecologically functional level throughout their range.

Appendix III includes taxa that an individual CITES Party may independently use to identify native species that are already protected within its own borders, but which require the cooperation of other Parties to prevent or restrict their exploitation. When a country lists a species in Appendix III, export from that country requires an export permit. There are no Appendix-III primates.

Special provisions exist for trade in specimens from captive breeding facilities that are registered with the CITES Secretariat. Facilities applying for registration must demonstrate compliance with criteria such as infrequent infusion of wild stock into the captive population and documentation demonstrating successful breeding to at least the F2 generation. Appendix-I species bred in captivity for commercial purposes at registered facilities are treated as if they are specimens of Appendix-II species.

A limited number of additional exemptions and special provisions exist under the Convention:

- Once a specimen has been exported from a country, subsequent re-exports from one country to another can be conducted with a re-export certificate, which can be issued more expeditiously than an export permit, as long as the specimens can be shown to be those that were originally imported with valid permits or re-export certificates.
- The treaty does not require additional permits for specimens transiting a country (i.e., not the country of export or import) if they remain under customs control.
- Specimens that can be documented to show that they were acquired before the Convention applied to the species concerned may be issued a pre-Convention certificate in lieu of any other permit.
- The noncommercial exchange of specimens between scientific institutions is also exempted from the normal permit requirements, provided both the sending and receiving institutions have been registered with the CITES Secretariat by the governments of the countries in which they are located. Once registered, an institution may make multiple shipments under a Certificate of Scientific Exchange according to established procedures.

In general, if a species is listed as endangered or threatened under the ESA, it cannot be imported or exported unless it meets certain conditions such as for scientific research or to enhance propagation or survival or, in the case of threatened species, for zoological exhibition or educational purposes. In such instances, findings of jeopardy (the effect of the activity on the survival of the species or population) and enhancement (how the activity will benefit the wild population) must be made. Also for threat-

ened species, the ESA authorizes the Secretary of the Interior to issue a regulation, commonly referred to as a 4(d) rule, which gives the Service additional latitude in implementing the ESA. There is a special rule for certain nonhuman primate species protected as threatened in the regulations at 50 CFR 17.40(c). The special rule exempts some species of legally imported macaques, langurs, and other species and their progeny from ESA coverage. It does not, however, exempt them from CITES coverage.

IMPLEMENTATION OF THE REGULATIONS

Inspection of shipments and compliance with the transportation regulations are enforced by the Division of Law Enforcement (Tel: 703-358-1949, http://www.le.fws.gov). The Division of Management Authority (DMA) (Tel: 1-800-358-2104, 703-358-2104, http://international.fws.gov) provides and reviews permit applications and issues permits for imports and exports. The Division of Scientific Authority (DSA) (Tel: 703-358-1708) is responsible for making jeopardy findings under the ESA and detriment findings under CITES, which it provides to DMA for consideration in issuing or denying a permit. It is also the office responsible for listing or delisting species under the ESA and CITES. Permit forms may also be obtained through a fax retrieval system by calling 1-800-770-0150 or 703-358-2348.

To obtain a copy of the Live Animal Regulations, contact: International Air Transport Association (IATA), 800 Place Victoria, PO Box 113, Montreal, Quebec, Canada H4Z 1M1, Tel: 1-800-716-6326.

The Toronto Zoo

William A. Rapley, DVM, MSc

PURPOSE AND KEY MANDATE

The purpose and key mandate of the Toronto Zoo include the following:

1. Emphasize the role of zoos in conservation, which includes the preservation of endangered plant and animal species and threatened natural areas. The exhibits emphasize zoogeographic habitats, the stewardship role of the human species in managing our planet, and the maintenance of biodiversity and natural ecosystems. Conservation is a major theme throughout the Zoo, including both public exhibits and educational programs. Graphics, interpretive signage, and labels stress this theme.
2. Provide direction to staff in the use of progressive animal care and management practices. The unit plans and co-ordinates exhibit renovations with other units in the Zoo. The curators oversee the animal and plant collections, including additions and dispositions, planning and construction of displays, husbandry techniques, and educational initiatives. The unit also develops and implements conservation projects that increase the Zoo's profile at the local, provincial, national, and international level.

Director, Conservation Biology and Research Centre, Toronto Zoo, Toronto, Canada

2001 KEY SERVICE ACHIEVEMENTS

- The Gorilla Rainforest project was completed and became a prime attraction. B&C staff contributed to the project team for Zoomobile circulation and to the planning team for Children's area.
- The orphan polar bear cubs were also a big media and public draw.
- Dr. William Rapley travelled to Taman Safari, Indonesia, to participate in the Indonesian Primate Conservation Assessment and Management Plan (CAMP). Under the leadership of Dr. Ulysses Seal of the Conservation Breeding Specialist Group of the IUCN (CBSG), this workshop brought together 85 participants from government, universities, zoos, nonprofit agencies, and regional parks, to develop an assessment plan for 40 species of Indonesian primates. Conservation management processes will be essential as it is estimated that 85% of the primate species in Indonesia will be eliminated in the wild in 10 to 15 years.
- Dr. Rapley and Paul Harpley participated in an Asian Bear expedition to Chirisan National Park in Korea. Afterwards they assisted Dr. Ulysses S. Seal of IUCN/CBSG and three bear biologists with an Asiatic Black Bear Population Viability Analysis (PHVA) and Habitat Workshop held at the Seoul Grand Park Zoo. This species is seriously threatened in Korea. Caroline Greenland joined the group, and a Phase II Workshop for the Masterplan and Conservation Plan was held by IUCN/CBSG in the second week. Dr. Rapley coordinated the conservation planning workshop section.
- Dr. Rapley and staff hosted a Korean delegation from Grand Park Zoo and the University of Seoul, Korea for 5 days. Detailed site tours emphasizing conservation and exhibitry were completed. Paul Harpley completed an exhibitry design workshop. Delegates were taken to the Royal Ontario Museum, the Royal Botanical Gardens, and Niagara Butterfly Exhibit. The project was to assist with the master plan and conservation plan for the Korean Zoo as part of the sister zoo relationship.
- Elaine Gabura applied for and received a Bursary for Advanced Specialized Studies from the Canadian Museums Association, to attend the AZA Population Management course at the School for Zoo and Aquarium Personnel in Wheeling, West Virginia.
- Dr. Rapley was invited to the City of Acapulco, Mexico, along with Councilor George Mammoliti, Chair of the Toronto Zoo Board of Directors. George has been working with community and city leaders to promote co-operative ventures and programs between Acapulco and Toronto. The Acapulco group included staff from the Mayor's office and Chamber of Commerce and leaders in the tourist trade and business industry in the city. The group is dedicated to improving environmental programs and infrastructure in Acapulco, which includes power, electric-

ity, sewage systems, water quality, and roads. Tourism and ecotourism from Toronto to Acapulco will be promoted. The ACA-Zoo run by the State of Guerrereo, located on Roqueta Island, was visited and assessed. An assessment of Roqueta Island, which is a National Park, and the nearby Coyuca lagoon, which needs conservation action and protection, were included in the study at the request of the Asociacion Ecologica y Prodefensa de La Isla de La Roqueta. Dr. Rapley also attended an environmental planning symposium for the State of Guerrero.

- In April, Eldon Smith gave a talk titled "Keys to Successful Port Practices" at the Air Animal Transportation Association (AATA) World Summit on Port Practices held in Toronto, from April 29 to May 3. He was also presented with the Robert D. Campbell Memorial Award in recognition of his outstanding organizational skills, expertise, and assistance to the industry in the transport of zoological animals worldwide.
- The Toronto Zoo hosted the second International Migratory Bird Day event in May, which included bird watching tours and educational displays.
- The Director of the Havana Zoo, Dr. Elsie Perez Dulong, visited the Toronto Zoo and met with Zoo staff to plan the CIDA project and Havana Zoo collaboration. Three-phase project to establish new exhibits, improve animal care, and work with conservation programs for endemic species has received further funding from CIDA and the Toronto Zoo.
- The Toronto Zoo hosted the first Environmental Week event in June, which included 20 outside organizations such as FLAP, Ducks Unlimited, CWF, FON, setting up displays around the Zoo site.
- Dr. Rapley served his third year as President of the Canadian Committee for the World Conservation Union (CCIUCN) and was part of the Canadian delegation at the World Conservation Congress (IUCN) held in Amman, Jordan, in October 2000. Dr. Rapley and CCIUCN hosted workshops for the Canadian Earth Summit Planning Group in preparation for the World Sustainable Development Congress to be held in Johannesburg, South Africa in September 2002 (R10 Biodiversity Conference +10 years)

2002 GOALS

The Toronto Zoo has the following goals for 2002:

1. Continue to develop and improve the programs in conservation and education as outlined in the new strategic plan;
2. Establish a CBSG Canada network;
3. Participate in the World Sustainable Development Congress to be held in South Africa in 2002;
4. Support Giant Panda Conservation efforts in situ and ex situ;

5. Provide support for conservation of lowland gorilla in situ; and
6. Support worldwide and Canadian efforts to control the bush meat working groups' activities in Africa.

Chinese Macaques—East Meets West

C. K. Hsu, DVM, PhD, MPH, and Ruishen Jia†*

China has developed and become one of the major breeders and suppliers of macaques for biotech-pharmaceutical-medical communities worldwide. China began the establishment of the rhesus (*Macaca mulatta*) captive-breeding program in 1978 and that of cynomolgus (*Macaca fascicularis*, of Vietnam/Cambodia origin) in 1985 for scientific research. The first export shipment of rhesus was in 1984 and cynomolgus in 1990. A total of 24 primate facilities are engaged in the breeding of either rhesus or cynomolgus monkeys or combined species. All primate facilities must register with and obtain licensure from the provincial government. An annual inventory of breeders must be reported to both provincial and central government authorities, which are responsible for the management and monitoring of the primate resources nationwide. Before 2001, three companies of quasigovernment status exclusively handled all exports of primates. With the recent Chinese WTO membership, beginning in 2002, it is a common belief that this commercial exclusivity practice should and will be removed. For each and every export shipment, the breeding facility will be carefully scrutinized by the authorities and must prove to be authentic from the captive-breeding program before a CITES is issued. All export-intended animals are subject to a 45-day period of

*Shared Enterprises, Richlandtown, PA
†Shared Animal Health and Technology (Beijing), LTD, Beijing, China

quarantine and tests including 3-TB (-), *Shigella/Salmonella* (-), and independently confirmed by the tests performed by the Animal Quarantine/Monitoring Bureau, which must then issue the Health Certificate for monkeys to be shipped abroad.

The production of macaques in China has increased steadily every year to meet both its domestic and export needs. The quality of macaques is generally very good, with a very low incidence of SRV, SIV, and STLV antibodies at ages 2 to 4 years. The accumulated viral serology data also indicate that the incidence of cercopithecine herpesvirus 1 (herpes B) antibody is significantly lower in rhesus than in cynomolgus macaques. In 2001, a total of 3802 rhesus and 8482 cynomolgus macaques were shipped from China to the United States, Japan, and Europe for biomedical research, a remarkable increase from that of 2840 and 6812, respectively, of 2000.

Since 1990, China has become an increasingly valuable and reliable source of both rhesus and cynomolgus macaques for international scientists. The increasing trend may very well continue for years to come. This optimistic view is based on the fact that (1) three of the five worldwide airlines available for transoceanic shipping of nonhuman primates from China to the United States, Europe, and Japan are Chinese; (2) increased production of improved quality of captive-bred offspring (6000 rhesus and 15,000 cynomolgus in 2001); and (3) few transportation accidents cited by receiving countries in the past decade. Three major exit airports in China, namely Beijing, Shanghai, and Guangzhou, have been used to handle all China-origin macaques and a significant number of transit shipments of cynomolgus monkeys of Vietnam and Indonesia origins. In 2001, approximately 190 shipments of 12,500 macaques of China origin and 7000 cynomolgus macaques of Vietnam/Indonesia origin were shipped from China to the United States, Japan, and Europe (France, Germany, Netherlands, Spain, Sweden, and the United Kingdom) via three Chinese air carriers: Air China, China Eastern, and China Southern. At this time, only Los Angeles and Seattle are used to receive macaque shipments via passenger or cargo flights from China. Chicago may be an excellent choice for receiving Chinese macaques using the cargo flights of two Chinese airlines. In Europe, Air China has carried primates to Amsterdam, Copenhagen, Frankfurt, Madrid, and Paris. In Japan, the three Chinese airlines have transported macaques to Tokyo and Osaka. Attempts have been made to establish and produce specific pathogen-free rhesus and cynomolgus macaques (free of herpes B, SRV, SIV, STLV); however, progress has been extremely slow in China.

Shipping crates with two, three, or four compartments, which exceed International Air Transport Association standards, are used to ship macaques. The crates are made of either hardwood or plywood. The two-

compartment crate made of hardwood is probably the strongest and most commonly used by Chinese primate facilities. All hardwood crates must be fumigated with ethyl bromide and certified by the government authorized facility as dictated by the agreement between US Department of Agriculture and the Chinese government to prevent the introduction of Asian longhorn beetles, which have caused severe loss of valuable hardwood trees in the United States.

The international transportation of nonhuman primates between continents remains a critical and unpredictable risk factor for both biomedical communities and primate breeding facilities worldwide. Its impact on the use of imported primates for scientific research is probably as significant as the impact on captive-bred animals and the conservation of natural primate resources. Scientific communities worldwide must deal and communicate with airline industry to avoid a severe interruption of research using primates. Fewer and fewer air carriers are willing to ship nonhuman primates in the air and to handle them on the ground.

All evidence indicates that the valuable resources of both rhesus and cynomolgus macaques in China will remain a reliable and important source for biomedical communities worldwide. The current status of Chinese macaque resources is summarized in Table 1.

TABLE 1 Current Chinese Macaque Resources

	Macaca mulatta	*Macaca fascicularis*
Natural resources	200,000	0
Captive resources	25,000	53,000
Breeding female	10,000	20,000
Production (weaned)	6,000	15,000
Breeding facility	19/25	16/25
Quality	Excellent	Excellent
Export	3802 (27%)	8482 (35%)
International transportation	Excellent	Excellent
Use	Increased	Increased
Risk	International airports	International airports
Trend	Promising	Promising
Resource potential	Very good	Very good

Session 6: Panel Discussion

Participants:
William R. Morton—Session Chair, Washington National Primate Research Center, USA
Nelson Garnett—NIH/Office of Laboratory Animal Welfare, USA
Jerry DePoyster—APHIS/USDA, USA
Michael Kreger—US Department of the Interior, USA
William A. Rapley—Toronto Zoo, Canada
C.K. Hsu—Shared Enterprises, USA

QUESTIONS AND ANSWERS

DR. BAUDOIN (Mario Baudoin, Ministry of Sustainable Development and Planning): This question is for the whole panel and if someone has an answer I will very much appreciate it. Quite simply, I would like to ask whether the US government can require US airlines to carry monkeys and if so, how. Is there a way for people here and in other related organizations to lobby? For three days now, we have discussed the need for monkeys and other nonhuman primates in research.

DR. MORTON (William R. Morton, Washington National Primate Research Center): I do not think there is any way that the US airways can be required to carry animals of any kind. That decision is purely theirs to make. It is basically an economic decision, and many factors enter in to how that is economically driven. I really do not think there is any kind of

lobbying that is going to force these airlines to take what, in their view, is a very, very small portion of their overall cargo with many associated problems. My opinion, based on what I know of the US government, US research interests, and certainly NIH-based research interests, is that there should be a concerted effort at the NIH government level to make a determination as to how this critical resource for the nation will be carried—whether international or domestic resources are needed to move this resource from research institution to institution. For a long time we thought (and I know I am not alone in this) that there should be some sort of central convening of the minds as to how we can approach this problem—in contrast to a private US airlines kind of direction. Now maybe some of you on the committee have other thoughts about that matter.

DR. RAPLEY (William A. Rapley, Toronto Zoo): I would like to mention only that we still have some arrangement with Air Canada because we have done so much with them. When we recently sent the mandrill to Los Angeles for the species plan, we were able to fly it to Vancouver and then transport it by vehicle to Los Angeles. However, with the black-footed ferret project currently in progress, we have been involved with chartering planes. One of the main reasons was to avoid absolutely any potential contamination of canine distemper at the airport. So we have gone directly from small planes from Toronto to Omaha, which we have done in a number of cases with species. Small charter has some potential there.

DR. BAUDOIN: I can understand the situation within Canada, but thinking of such countries as Indonesia, Barbados, St. Kitts, and so on, there is a great difference to the airlines of a monkey meant for a zoo for education and a monkey for biomedical research and to save human lives. The point here is that big difference to the airlines.

DR. HSU (C.K. Hsu, Shared Enterprises): About 4 to 5 years ago, even a US carrier originally from China did carry some nonhuman primates from China to Japan. The latest was Northwest Airlines to Los Angeles, which for some reason they recently stopped. That was long after. They do not even carry animals domestically, obviously, and right now they refuse to carry any nonhuman primates from China to any part outside China.

PARTICIPANT A: Dr. Garnett, you mentioned in your speech that there is a report with which I am not familiar, that had some suggestions/recommendations about relieving some of the current regulatory burdens. One of them was that permits would not need to be required for species that are immediately threatened with extinction. I have some follow-up questions, but I was wondering if you could elaborate briefly on what species and in what fora those permits would no longer be required.

DR. GARNETT (Nelson Garnett, NIH/Office of Laboratory Animal

Welfare): That language was very convoluted and it was coming directly out of the Mahoney Report. Essentially it was asking Fish and Wildlife to not extend their regulations beyond what the actual law required. The specific language, although difficult, is available on the NIH website (*http://grants1.nih.gov/grants/policy/regulatoryburden/index.htm*). Essentially it is an overarching report that was mandated by the NIH budget hearing for NIH's funding. It involves an extensive view of five different areas of regulation considered to be burdensome: human subjects research, animal research, conflict of interests, and a number of hazardous waste disposal issues. The report includes the group's recommendations.

PARTICIPANT A: Are these permits that would no longer be necessary the CITES permits for importation of species, which are threatened/immediately threatened with extinction?

DR. GARNETT: These were proposals made to NIH about issues that are not regulated by NIH.

PARTICIPANT B: I also am confused because it seems to me that the NIH recommendations, therefore, would be to the Fish and Wildlife Service directing them not to comply with CITES because Appendix 1, species under CITES, are by definition those threatened with extinction. I am confused because it seems that the recommendations would be to direct Fish and Wildlife Service and to not comply with its obligations under CITES.

DR. GARNETT: I do not think that was the intent. There may be some Mahoney Committee members here who could shed some light on that.

PARTICIPANT C: They would apply only to scientific institutions that are currently given general permits, but only for herbaria and for museum specimens. However, it would apply to research specimens, and general permits are given to institutions like Harvard University. So there are some dangers, but there are also some savings in time.

PARTICIPANT B: I would like to argue with the point about the dangers. I believe it is incredibly dangerous to give a blanket permit, especially CITES, to an institution for wildlife. It is particularly dangerous without defining what "threatened with extinction" means because, of course, there is a definition of threatened with extinction under the Endangered Species Act. There is also a different one under CITES, and a different one under IUCN criteria. I believe these distinctions are quite important. Because I was not on this Mahoney Committee, I wanted to point them out.

PARTICIPANT C: We are shipping animals from Israel to Europe. In my opinion, the first reason airlines give for not transporting animals is the personnel involved, not the business policy. At least in our region a company will ship anything. I can tell you that from my experience be-

cause Israel does not have any possibility of shipping animals via ground. We sued our national company in court and we won. It has not yet ended, but we are glad that, for example, the park and sanitation organization in Israel joined us in court and stated that this is important.

I want to raise another issue connected to this—the welfare issue. I have heard in many government institutes that we are breeding animals in England and other places because we want to be sure we will have the supply in the future. Why then do we send these animals on a 5 hour flight and we put them in an indoor facility for 6 months a year and not in the temperature they like to live? No one commented about this welfare side of the problem. No one said anything about this complaint that basically they must be shipped to a distance 10 times farther when, for example, if we chose this because it was cheaper, we would be criticized all over the world. I think we must be much more decisive about transportation. If a company will ship goose liver and food made from animals, why will they not ship something that has a benefit for all of humanity?

DR. MORTON: You bring up some interesting points. From the US point of view and again from a point of view I can speak to in terms of the primate center's program, NIH, I believe you are seeing a gradual movement of breeding colonies to more appropriate areas of the country in the more southerly areas of the country, where it is a better environment for the monkeys themselves. Certainly it is a more productive environment and, speaking personally, a much improved environment in terms of health and well-being for the animals. However, it does bring us back to the problem of how to ship these animals reliably and in a timely fashion from those breeding facilities to the areas of use, the research facilities. That is a big problem, and I think it is part of this panels' overall problem.

DR. GARNETT: I think that the proposed study would be an appropriate vehicle for addressing a number of these problems and infrastructure recommendations.

DR. BAUDOIN: This question is for Dr. William Rapley. In Bolivia, we have 1,300,000 acres of protected areas managed in 1990. We have 15,000,000 now, but half of our country is being called "ancient forests." I attended the discussion at The Hague on this theme at the convention on biodiversity. The discussion has been dominated by the endangerment discussion, particularly in the last meeting in Montreal in September. We have gone from 0 in 1996 to 1,000,000 acres of certified forests now; however, we cannot accept that the ancient forests will be dedicated only for protection because that means 50% of our country, and we have a great many people living there. They do eat things and use things, and it is the only choice they have. To do nothing with the forest, the forest will be replaced by rice fields. I think that in the discussion on these issues, sometimes because only one aspect is stressed, you may have the opposite

effect. I wanted to mention this because in your talk, I think there was the message of the local people's usage of the forest in a destructive way, which I do not think is what you wanted to convey.

DR. RAPLEY: I think that is a good point. I did not really have the time to explain things. IUCN stands for sustainable use of resources and everywhere in the world we work with the endemic people, whether it is in the Arctic in Canada or in any country. You have to look at each resource and identify the areas that must be protected. The *Macaca fascicularis* projected world population is 20 million according to this, which is probably not very accurate, but we know it is a very low-risk population and not a problem. Let us say the 5000 to 7000 *Macaca fascicularis fuscata* that are found in less than a 10 km^2 are in danger. We need more studies of all of these species and the biodiversity and the monitoring to identify which areas should receive the most protection and which areas are perhaps not as important. Working with the people in the forest is extremely important. Dr. Mittermeier, I am sure, will say a lot about this tonight because we will extract wood; people have to eat; it is a way of changing the way they do that. If you take the wood and carve it or make things, then you send it out, rather than shipping the raw product (Canada is not successful at shipping the product somewhere else to have it made into a product). Such a process is not sustainable use as much as it could be.

In the villages, in Central Africa they had a tradition of shooting the gorillas and chimpanzees cutting the thumbs, and putting the thumbs on a necklace around the child's neck to protect it from evil spirits. A tremendous amount of work is required to attempt to change that tradition and to have some of the species that are common there hunted on a controlled basis. They are going to have some farming and other activities, but hopefully they are not going to kill chimpanzees and gorillas. In every place, there is detailed work required to address every situation and define it, and I admit that we have not spent enough of our resources to really study that need. However, we are working to balance the situation as much as possible.

So there are different ways of doing things and as you know, IUCN promotes selective harvesting. I agree with the approach not to protect everything because it does not work.

PARTICIPANT D: The bush meat crisis has become a hot topic recently. It is often a topic in the *Journal of Conservation Biology*, and some of the reports we are getting with the work we do with the great apes conservation fund and African elephants fund indicate that there are multinational and national companies coming into some of these primary growth forests. They are hiring locals, but they may not be feeding the locals. Even though we know that locals have for years subsisted on some

of the wildlife that is endemic to the area, it has gotten so bad lately that there is a new term called "silent forest." We are finding that dikkers and other small antelope as well as birds are disappearing. People are eating the reptiles and so a lot of the fauna is disappearing at a really alarming rate.

DR. BAUDOIN: What are you going to tell people in the forests because I do not think it is just going to go away? No, I think that it is a recipe that you have to think about.

DR. MORTON: If possible, I would like to direct these questions more to transportation.

DR. MCGREAL (Shirley McGreal, International Primate Protection League): Speaking for transportation, I would like to address both Drs. DePoyster and Kreger and possibly our friend from China, Dr. Hsu. As you know, Dr. DePoyster, you do not have any real law enforcement through criminal or misdemeanor prosecutions but must work through administrative courts. I would like to commend Dr. Kreger, along with Special Agent Kirby and all the special agents, for their April 9th indictment of a major US importer, LABS of Virginia, and its president, for 12 major counts these including eight charges of shipping infant monkeys. The charges are eight felonies and four misdemeanors for shipping 3- to 4-week-old infant monkeys, which was totally outrageous. I am sure that all of the African bodies are very pleased also to see this indictment. The senior felony charges were for the importation of captive—reportedly captive—born animals that we in fact allegedly caught in the wild. Now we have heard numerous reports from anonymous Chinese sources at the International Primate Protection League that some of these monkeys coming out of China are, in fact, wild caught in Vietnam and laundered into the United States. I understand there is some sort of on-going investigation. However, I would like to ask whether you think it is possible for these huge numbers of cynos to be captive born, or is there some laundering going on?

DR. KREGER (Michael Kreger, US Department of the Interior): We hope there is no laundering going on. We do the best we can with our permits to check for legal acquisition to make sure these animals come from where they are supposed to come from. Very often we are in touch with other scientific authorities or other management authorities from the range countries.

Again, we can also talk to people in the zoo community or people who are out in the field to find out exactly what is going on in the range countries. Sometimes things do get in that are not supposed to come in, or people may fill out a permit form to give us the information that they think we want to hear, but we have law enforcement check into those kinds of things.

PARTICIPANT E: My question ties in with capture for transport to the United States versus the bush meat issue. I would like to ask Dr. Rapley to explain to me what the difference would be if an animal were CITES Appendix 2 listed and that animal were captured for bush meat, which you have called disgusting (but which to me seems more of an ethical judgment than a population-based assessment) versus transporting that animal to the United States to be used for research purposes. Could you simply explain what the difference would be from the zoo community's perspective?

DR. RAPLEY: The question is difficult, and I agree there is perhaps no easy answer. The whole idea is that we should try to protect in the wild those animals that are extremely rare. If we cannot because they are under pressure and must be rescued or moved and put in captivity, because the habitat is decreasing so rapidly, we must consider that information. For example, of the 40 species in Indonesia, many are recommended for captivity because otherwise, they will be gone as species.

Sometimes we place animals in captivity if they are endangered to conduct research on them for their own purpose. In other words, we learn how to breed them or learn about their genetics, learn about their potential for reintroduction, and things like that. So there is a wide array of different things that go on.

In Indonesia and other countries, if something is not done, there will not be any gorillas or chimpanzees in 10 to 15 years in the wild; they will be gone. It is very scary; something must happen.

I once worked extensively with a World Wildlife Fund traffic person who is based in Toronto. He showed me pictures of a transfer station in China where things are accumulated for shipment to all parts of the world for food. There were soft-shelled turtles, reptiles, and huge boxes and mammoth collections of these types of things. It seems to me that at this rate, there will not be very much left in the wild.

What I am trying to say is that I am not against biomedical research. I realize the benefits. I worked in the field directly for 8 years, and I understand why these things are important. I am just saying the really endangered species need protection and that the captive breeding such as the vervets in St. Kitts and the 20 million cynomolgus monkeys are not threatened. They are bred in captivity and they are used for biomedical research. I do not have a personal objection to that system. Nevertheless, there is a whole range of things to consider within the system.

Session 7

Unresolved Issues

Session 7: Panel Discussion

Participants:
John L. VandeBerg—Session Chair, Southwest Foundation for Biomedical Research, USA
Christian R. Abee, University of South Alabama, USA
John Hearn, Australian National University, Australia
Hilton J. Klein, Merck Research Laboratories, USA
William R. Morton, University of Washington, USA
John G. Vandenbergh, North Carolina State University, USA

QUESTIONS AND ANSWERS

DR. VANDEBERG (John L. VandeBerg, Southwest Foundation for Biomedical Research): I reflected this morning about the proceedings of the workshop, in which there has certainly been one recurring theme, which Dr. Hearn introduced and probably all of the speakers reiterated: that we do have a crisis and that actions are urgent. The most obvious component of that crisis, of course, is the shortage of nonhuman primates for biomedical research, especially rhesus monkeys, at a time when the opportunities for solving global health problems are far greater than they have ever been in the past, partly as a consequence of the revolutions in genetics, genomics, and molecular biology. This is also a time when entire societies of some developing countries are being destroyed by AIDS, superimposed on other infectious diseases that impose a huge burden of

morbidity and mortality in many of those countries. However, the basis of crisis is much more complicated than simply a shortage of monkeys.

We have heard about many other aspects of this crisis at the workshop. We have heard about insufficient infrastructure for breeding monkeys, that more monkeys could be produced if infrastructure were expanded. We have heard about insufficient infrastructure for conducting experimental research, particularly as we have new needs with the emerging infectious diseases and the bioterrorism initiative. We have heard about a shortage of veterinary pathologists and laboratory animal veterinarians, trained to work particularly with nonhuman primates. We have heard that species other than rhesus are poorly characterized, including many of their basic biological characteristics and specific characteristics that are pertinent to their use and potential as alternatives to rhesus as models for some diseases. There is also a lack of reagents for many of those species.

We have talked about difficulties in transportation. It is difficult not only to get monkeys transported around the world but also to have them transported on longer journeys with more stops. These journeys are certainly detrimental to the welfare of the animals.

We have heard about regulatory burden: too many regulations and too little flexibility are impediments to research and to establishing breeding colonies in some other countries.

We have heard about problems related to conservation of nonhuman primates and have discussed strategies for aiding the conservation efforts in tandem with increasing our biomedical research capabilities. We have also heard some encouraging news about ramping up the supply of macaques from captive bred colonies in China and Mauritius, the availability of vervets from St. Kitts, and the potential of producing rhesus in Nepal. We have heard about contributions to the conservation cycle in Mauritius and St. Kitts as a consequence of the supply of animals for biomedical research. We have heard about progress in the development of SPF colonies and the establishment of Chinese-origin rhesus as potential alternatives to Indian-origin rhesus for AIDS-related research. We have heard about progress in genetic and genomic research with nonhuman primates, which will accelerate progress toward preventing human diseases.

So the questions of this session are, where do we go from here? What are the unresolved issues that have been raised at this workshop, or perhaps have not yet been raised or need to be raised in relation to the issues we have discussed? By what mechanisms can we proceed in the months ahead, perhaps over the next few years, to resolve these issues?

I would like to ask Dr. Hearn, who began this meeting with the superb overview of many of these issues, if perhaps he would comment,

having listened to these last 2 days of deliberations, on what he sees as the unresolved issues.

DR. HEARN (John Hearn, Australian National University): Thank you very much, Dr. VandeBerg. Ladies and gentlemen, I find a sense of excitement, having been present here in the last 3 days at the research initiatives, the infrastructure needs, the potentials for really good new science and for conservation, as well as the prospects of partnerships both within the United States as a national program and internationally. In recalling the theme of my comments to you earlier, I would simply like to highlight a couple of those issues for your consideration, again, starting with a sense of priorities and ending with a sense of partnerships.

I believe that we have been privileged to see some important new data at this meeting. In a sense this meeting is unusual. It has given us a reality check on a number of areas of primatology and has given us a chance to look forward. I would like to thank ILAR, Dr. Joanne Zurlo, and Kathy Beil again because I think an important meeting like this might be restaged as a strategic reality check every 2 to 3 years, perhaps in conjunction with the Primates Society.

In itemizing a few items and endorsing the ones Dr. VandeBerg mentioned, I would describe the issue of AIDS and the need for primates for AIDS as a crisis. However, I think we need to see it in context. It is also a crisis of diminishing the abilities to develop other fields. I was not entirely convinced from what we saw that there were major problems with the Chinese rhesus in terms of the study of AIDS. It is necessary to look at both the Indian and Chinese rhesus. I think we have tended in the field to become almost obsessive about the rhesus for obvious reasons.

We have so many more data, fundamental data, on reagents. I endorse the issue you raised, that we really need to make an effort for the reagents in a few other species to retract the spotlight from the rhesus as a limiting factor. I will not repeat the areas that I discussed before, but I was impressed with the work now coming through in genetics with the potential for functional genomics and the ability to define the animal much more clearly. We can define required traits for experiments and reduce the number of animals, in many cases, by having more defined approaches. Likewise, from applications of colony management and studies in the field of groups of primates, endangered or others, we can get a faster assessment of their endangered status and the potential for their breeding as a result of that crisis.

I believe the laboratory and field are currently overworked and overfocused in our own questions. It is necessary to survive, to publish, and to deliver the quality we have in each of our fields; however, I wonder whether we should be spending a little more time thinking about the laboratory field interface. There is a lot of human primate interface with

the issue of emerging diseases, particularly in some areas of the world such as the Amazon, Congo, or Indonesia. In those areas there are existing primate facilities nearby in Africa or South America or Asia that can partner with those centers to develop our knowledge base of potential emerging disease and the epidemiology might be required. I think with clear focus on particular questions in virology and other zoonoses, we might learn a great deal.

I was also encouraged about the new initiatives to set up cell banks and develop stem cells from origins other than embryonic ones. I think we might hear about those initiatives shortly.

Obviously, the alternative issue to the rhesus is very important. I suggested in my talk two or three species from each of the major continents that might provide the alternatives both in general laboratory primatology and also in niche species, such as aotus for malaria and some of the ones we heard of in the program. I was concerned and rather surprised to learn that the NIH budget has doubled in the last 5 years whereas the primate and special centers' budgets have remained relatively flat. While I appreciate that much of that money is targeted, there is a question as to whether the primate centers are participating fully or able to deliver the best science. It seems strange to me because I know the quality and the performance of the primate centers, including the national and specialist centers. I hope that trend can be reversed, which is necessary in terms of equipment, PC, three or four facilities, and so forth.

To repeat another point I made earlier, we need to put more funding into special targeted field studies in the species that are utilized principally in biomedical research in an effort to understand more of their natural biology. It is a sort of dream that if you put 2% aside from the budgets into the general fund, which would be peer reviewed and accept only top quality work, I believe we could enhance the field as a whole: 2% probably would become $4 or 5 million. If industry, as major users of primates, also were to match that amount at a level of about 2%, the total could build up to about $15 million. You could make a major impact on conservation in captivity and in the wild of primate species in that way. That approach gives us, as biomedical researchers, greater power and understanding of the primate species we study and of ourselves—for industry. Although it may not be immediately attractive to the industrial culture to do such a thing, I think it would pay off rapidly.

So the state-of-the-art issues are of genetic definition, of clearer transfer of research into the human now that we have more noninvasive systems, and of international collaboration. Such partnership is critical because all of us are very pushed in our own fields, and we need a meeting such as this as well as follow-up action to form those partnerships. It all comes down to practicality and people understanding each other. If the

primate centers, and I include the special centers, each had one or two partners elsewhere in the world relevant to their specific fields, I think we could really see a "rising of the tide" for everyone in the field.

DR. MORTON (William R. Morton, Washington National Primate Research Center): I would like to summarize what I think are a few of the unresolved problems that require our continued work. They are in no particular order of importance, but they all are significant.

First, there are huge problems regarding transport of nonhuman primates, which have not been resolved and are getting worse. We identified a few airlines in Dr. Hsu's presentation, and the primary carriers are all southeast Asian now. We can rely on very few domestic airlines to carry primates in the United States. I think we need to discuss this area and we need to resolve in some way whether we will have reliable, timely, quality types of transport capabilities.

Second, there needs to be more investment in conservation-oriented programs within the research field. Conservation efforts Dr. Mittermeier described last night should be increased within the established research arena, such as the national primate center's program. We have great difficulty in convincing site reviewers and grant reviewers of the need for that commitment because there is such a great focus on the end product, of specific research programs that focus on molecular biology of the AIDS virus and the molecular advances in neurosciences and imaging and all of the things we know are important. Unless we pay attention to and invest in the basics of conservation and field biology, I think we are not going to be successful in the future.

Third, we all have focused on the need to look at other species. We all know there is a great shortage of the Indian-origin rhesus monkey, so we need to look at other species; but to do that, we need to educate the researchers. Everyone in this room is of like mind, for the most part. It is the research community, the people who use these animals, we need to convince that they can have alternative approaches to other species, other methods. That is the real crux of this whole argument, and we need to communicate with those people in some way. They need to be sitting here in this room and they are not. I think that is the major problem on which we need to focus.

Lastly, we have heard again and again that there needs to be a larger investment in the infrastructure of facilities that maintain and handle these primates, and we should be in the forefront in thinking of the way these animals will be handled in the future. That positioning calls for a huge investment in new facilities, rethinking and retooling the way we house these animals, which can only come from a commitment from NIH and other like-funding agencies. I think we need to talk about those unresolved issues, not just here but into the future.

DR. KLEIN (Hilton J. Klein, Merck Research Laboratories): I believe this meeting was very important because it showed that there is a high level of cooperation and willingness to discuss these issues in a forum like this on an international basis. This is a very positive sign. Like Dr. Hearn, I am hopeful and excited, and I think this cooperation gives us the power to deal with some of these problems that each one of us has dealt with in our individual sessions. From the microbiology point of view, as it relates to Dr. Morton's comment about infrastructure, there is a very important need for standardization, not only for the tests we would use to define a specific pathogen animal for biomedical research but also for the test methodology in the reagents. That step is really the next one in all of this.

The other aspect that has changed from the past in our world of using monkeys is a very important need not only to integrate the microbiology to find the SPF monkey but also to learn how to maintain the microbiologic status when we now must profile them genetically. Combining this profile with the behavioral aspects in an integrated fashion to craft an SPF colony and redefine it is an important aspect of biomedical research's future. I think it is one of the big challenges that is often overlooked. It is very obvious, but not stated.

I think with the resources, conservation aspects, and the true clear-cut need, this is an exciting time for us. The monkey is really a strategic element of biomedical research, and I think we have the resources to make advances for society as long as we give it the priority that it requires. I think integration and cooperation are essential to making this work.

DR. ABEE (Christian R. Abee, University of Southern Alabama): I will begin simply by reminding everyone that 1978 was the last time we created a national primate plan, when we looked at all of the primate resources and how they should be used in biomedical research and then tried to think forward. Over the years that I have been in this field, I have found that funding agencies that support the kinds of things we are talking about wait to act until there is a big problem, rather than addressing it as a prospective thing. That approach creates a serious problem when it comes to primate resources. You do not instantly breed up a national supply of macaques or of neotropical primates; it takes years to do that. If you wait until you have a serious problem, you have waited too late, and it does have a very negative impact on biomedical research. So part of what I hope we can do with this meeting and perhaps meetings in the future is to try to guide our funding agencies and make them aware of what needs to be done before it is too late in some cases.

One other thing that we need to think about doing is to look at alternative species. Although there is very little or no funding to explore alternate species, it is very expensive to do primate research regardless of the

species that you are working with. Shortages of primates for research exist not only with rhesus monkeys but also with squirrel monkeys and aotus owl monkeys, for use in malaria research.

I also believe that we need to work harder on partnerships with source countries in developing primate resources. Both from what I have heard at this meeting and from what I know from the field, very positive things are going on in Asia. However, much less is being done in South America, for instance, with neotropical primates, aside from the Peruvian primate project. There really is almost no development of primate resources in South America.

I will close by thanking the speakers who came from six continents to talk with us about primate conservation and supply and to share their thoughts. I believe this is an excellent start for us to begin thinking about how we can strengthen partnerships with our colleagues around the world, and particularly in source countries.

DR. VANDEBERG: I think I heard a potential recommendation when you talked about the national primate plan. I had forgotten how long it had been—almost a quarter of a century—since we had a national plan. It is shocking to me that we have been going from year to year, from study section to study section, with little continuity and with no real comprehensive plan to address the potential problems and potential opportunities of primate research. What do we need to do years in advance of having the resources that we need because of the long generation time and the few number of offspring produced by nonhuman primate species? Are you suggesting that we consider a national primate plan?

DR. ABEE: Yes, I think a national primate plan is important because it will not only help us understand the need for primates in research globally but it will also provide guidance to funding agencies as well.

DR. VANDEBERG: It seems that many of the issues we have discussed at this workshop are very complex, and clearly we are not going to solve the problems in a couple of days here. We are going to uncover many of the problems, but it seems that many although not all could be handled well in a plan that is carefully developed over some period of time by a diverse group of experts.

DR. KLEIN: As Dr. Abee and I discussed some time ago, and based on what we heard yesterday about conservation and what is going on in threatened parts of the world, I would suggest calling it an international primate plan. I think it is more appropriate in this community and in this era than a national plan.

DR. VANDEBERG: I think you are absolutely correct. It has been very clear from the discussions at this meeting that the world is very integrated in its use and supply of nonhuman primates, and an international primate plan makes much more sense than a national primate plan.

DR. HEARN: I agree that a plan of this sort is vital because it opens opportunity. I suggest providing broad guidelines for 5-year and 10-year objectives and then proposing activities for the next year or 2 years to work toward achieving those goals. Then you revisit it after 2 years and update it.

DR. VANDEBERG: The audience should feel free to comment on desired aspects.

DR. RAO (A. Jagannadha Rao, India Institute of Science): This meeting is a very useful for my understanding of the problems. I really appreciate the idea of an international primate plan. As all of you know, primates are time consuming to breed in sufficient numbers for our use. One way I have been working to impress my country is to attempt to combine programs, as in Indonesia's and Washington University's collaborative programs, so that the infrastructure in source countries can be combined. The standards can be acceptable internationally. Collaborative programs can be carried with the conservation biologists so that certain things are immediately evident. To allay any suspicions or fears, the best thing is to have immediately visible results as far as certain endangered species are concerned.

Perhaps programs that are relevant between particular countries, such as between the United States and India, can be developed under improved conditions as a first step so that the animals can be used as they are needed. Transportation problems would be solved because the plan would be economically viable, and maintenance could be improved through initial goals to soften the rigid conditions so that eventually, perhaps 5 or 10 years from now, conditions are improved and export is possible. I believe the shortage of rhesus monkeys, macaques, and vervets, can be solved.

DR. BECK (Jeanne C. Beck, Coriell Institute for Medical Research): I want to thank the organizers for an extremely interesting several days. There is a lot of information that we can take home from what has been presented. I want to tell you that under contracts from the National Institutes of Health and the National Science Foundation, Coriell establishes and distributes cell cultures for use in research. From the discussion of the last few days, it would seem that as nonhuman primate resources become scarce, a more vigorous effort to bank its cells and tissues from these animals is essential.

Although cell cultures could not be used for all of the studies suggested, they would be useful for genetic studies, comparative and functional genomics, and aging and normal biology. From the tissues we have obtained from the Washington National Primate Research Center, we have been able to establish differentiated cell lines and to isolate what I consider something very exciting—multipotent adult stem cells from both

fat and brain. Dr. Hearn mentioned primate partnerships, which I think we should consider banking tissues and establishing cell lines.

Furthermore, in countries where export is precluded with the appropriate permits, it would be possible to collect samples and establish cell lines from these animals. Although this method will not solve the shortage, it may alleviate some of the shortfall.

I would like to mention that we recently obtained funding from the National Science Foundation to set up a bank of cell lines from primates. We hope to have a male and female from each species and then also to include some species in depth. One of the very important things I think this grant permits us to do is to work with range countries and to do capacity building. I would urge you to consider banking these incredibly valuable animals for the future.

DR. VANDEBERG: Thank you for informing us about those activities. Certainly at our primate center we have discussed in the past the idea of establishing and maintaining cultures from some of these pedigree baboons, especially some of the original founders. Some of them are dead now. Although 15 or 20 years ago we thought we had collected enough blood samples from them to have enough DNA to last as long as we wanted, we now find ourselves short of DNA, with a loss of that opportunity for future genetic research. If we had immortalized cultures or even had established cultures from those animals, it would have been very valuable to the future of nonhuman primate research. Our center would be delighted to work with you on that initiative, and I expect the other centers would also be so inclined.

DR. ERVIN (Frank Ervin, McGill University): I think that is a wonderful idea. We do have cultures on all of our animals that have been in our hands. However, I want to mention something quite different. I am slightly puzzled by something Dr. Morton said. I suspect that the investigators should not necessarily be at this meeting but instead should be subject to you telling them something about what they should do.

I have a proposition. I will donate up to 20 animals, if you will pay the transportation, to any approved project that will substitute a species different from a rhesus, which in your scientific judgment does not require a specific species. I happen to know that in Washington there are a number of such projects in which very competent scientists are using whatever monkey they learned about during graduate school.

DR. MORTON: I think that is a great comment. I agree with what you said, that these researchers need to hear more of this. Although they hear it from us and from people who are not here (e.g., veterinarians) they tend to take it less seriously than if they were hearing it from a national or international gathering like this, which is focusing on these problems. They need to understand these problems. I think that the point you made

about trying other species in what might be very acceptable ways for specific experimental projects is something we would very much like to do. However, as I think someone has pointed out, these programs are expensive to operate, and there is often a lack of funding for infrastructure to support them. Money is necessary to support these exploratory investigations to compare and contrast different species as to when and how they can be used. We saw a great example of it yesterday. Dr. Marthas compared and contrasted Indian-origin Chinese rhesus and made her conclusions from that. We need to expand that approach and look at the African green, the fascicularis, and other primate species that might have greater availability not be as threatened as the rhesus monkey. I am in total agreement with you and would very much like to work together.

DR. VANDEBERG: That offer is extraordinarily generous. To follow up on what Dr. Morton said, I believe that funds should be dedicated to that purpose if alternatives are to be seriously tried. We are never going to get funding from a study section to try out a new species. Unless there is an administrative commitment to support the development of new species as models, it will not happen. The investigators do not have the money to do it and they cannot get the money to do it.

Your offer is certainly generous. Perhaps we will stimulate some further thought on how we can obtain the other resources needed to explore the vervets as well as some other species as potential models.

DR. ROBERTS (Jeffrey A. Roberts, University of California—Davis): Relevant to the Chinese-Indian project that was done at Davis, I want to mention that it was subsidized in part by a specific primate center base grant to look at the comparative biology of these two species. That subsidy is a critical element of the base grant function.

Additionally, in terms of educating the investigator or getting the message out there and also in the context of developing an international primate plan, I believe it is important to obtain some consensus or body of information from the different NIH institutes about primate demands and model leads. I know there have been some efforts along these lines, but different meetings seem to focus on different demands (e.g., NIAID on AIDS and NIA on aged primate models).

I think that if we had greater consensus or coordination from NIH regarding the expected future primate demands, we would benefit in terms of planning and the opportunity to educate program people at the different categorical institutes about the availability of different species. I think NIA has been successful with studies that have looked at biomarkers of aging and the aged rhesus macaque. It has alerted them to the resources available in the aged baboon, the aged squirrel monkey, and many other species available for aging research. Those resources also offer the opportunity for comparative studies that are sometimes the most infor-

mative. I think as we look at this, we will definitely get more of a sense of a demand from the community. I also think that educating the program officials about the availability of African greens, cynos, and New World primates will be beneficial in interactions with investigators.

DR. VANDEBERG: It seems to me that Dr. Roberts' suggestion is an excellent idea for one of the potential charges to a committee developing an international primate plan. Someone must assemble that information. It takes some effort, and it seems to me that a committee could ferret out that information from the many institutions of NIH. Certainly that could be one of the components with which to charge such a committee.

DR. ROBINSON (Jerry Robinson, National Center for Research Resources): Along those lines, I would like to bring to your attention that we have received a request from the Secretary of the Department of Health and Human Services to form an interagency committee to review the total needs for nonhuman primate research. That committee had its initial meeting 1 or 2 weeks ago, with representatives from CDC, Department of Defense, and FDA as part of the committee.

DR. VANDEBERG: Is that group going to do what Dr. Roberts was suggesting and learn from all the NIH institutes as well as other government branches what the proceed needs are for nonhuman primates?

DR. ROBINSON: Exactly. Melding into that information is the NCRR's recently completed survey on which I reported, indicating that approximately 13,000 nonhuman primates were used by NIH grantees in 1999, and we know that need is increasing. In addition, I would like to mention that even though we have not publicized this information, we at NCRR and the Office of AIDS Research have formed a small working group, which is convening today to look at this very issue. We want to look at the use of Chinese versus Indian species in AIDS research. We are also trying to look at alternative species to alleviate the pressures on rhesus macaque. We are bringing in some experts, many of them are here at this meeting to help us look at the possibility of using the baboon, the pigtail, the vervet, the cynomolgus, the squirrel monkey, tamarins, and the marmoset as alternatives species in other research. This meeting is open and will be in Room 150 beginning at 1:00 pm.

DR. BAUDOIN (Mario Baudoin, Ministry of Sustainable Development and Planning): I would like to endorse what Dr. Rao said regarding the need to work on partnerships to be able to build permanent institutions within source countries. The result will be continuity and programs that will shift perceptions. In addition, for instance, we want to give permission for squirrel monkeys to be exported from Bolivia. We have a request for 1500, and if we want to do things well, we should have an idea from where in Bolivia they will be coming and what the impact of the extraction will be in Bolivia. We do not have the basic data about popula-

tion densities within Bolivia to make that recommendation. It would be helpful to have even a very small investment and some basic field research about population densities in the source countries. In the case of the squirrel monkey, even a minimal amount of genetics will be informative because we do not know from precisely where the original squirrel monkeys came that were exported in the 1980s and have been good models. Making a small investment in those things would be a great contribution to having programs work well.

PARTICIPANT: First, I would like to congratulate everyone on this eye-opening meeting. I agree with the people who are from the less developed regions of the world. I think there is a difference between saving and investing. I know that the stock markets are not doing very well these days. Essentially, if we look at the future, and the less developed regions of the world, we see that those of us in countries that have the monkeys also have a very large gap in technology. We cannot contribute to vaccine development or science unless we choose to be partners. We have to start addressing those questions if we want to invest in the future and be partners in research as well as in supplies of material.

The Caribbean region is particularly important. Not only in South America (and I am in full agreement with what my Bolivian colleague said) but also in the Caribbean, the second highest incidence of AIDS exists. Obviously some vaccines are going to be tried there in monkey experiments. However, those countries should not be used only as human resources for testing vaccines but also maybe as intellectual partners in the science development. I am sure there are many Latinos who are quite capable of elaborating on that subject.

We have learned from yesterday's lecture and from comments here that we are in a global world and are all partners in science. I believe we need more integration, dialogue, and inclusion. I think the time is right for forming international committees for transportation issues and for future events. We have an AIDS epidemic now and we will have other epidemics in the future. No one expected bioterrorism, and it is with us now for years to come. We need to look at the future and at investing in particular areas not only to consolidate resources but also to develop infrastructures to protect our target locations against bioterrorism.

DR. SEIER (Jürgen Seier, Medical Research Council): I would also like to congratulate ILAR on a very excellent workshop. I would like to reiterate the call for forging partnerships with facilities in source countries. From the point of view of my own facility, I certainly cannot supply anyone with primates because it is not the mission of my facility. Nevertheless, it is the only facility of its kind in South Africa. We have several hundred pedigreed vervet monkeys whose importance we have heard

throughout this workshop. They are tested for a variety of micro-organisms and are pathogen free. They are looked after by staff who is qualified as in the United States and Europe. We have scientists in the facility whose postgraduate research has been entirely on vervet physiology. We have consultant veterinarians and pediatricians. The animals are in excellent quarters according to international expectations. We have an environmental enrichment program.

The benefit of these important partnerships is that we have a track record of research. The profits of contract and corporate research are not put into some corporate structure or bureaucracy, but are reinvested in the animal facilities. For example, I am employing a full-time zoologist to provide environment enrichment and research and to develop vervet-specific enrichment. We are looking at stress levels in different housing conditions, for example, and the studies are entirely funded by contract research. Unlike people wondering whether or not we can use other species, we have been using the vervet monkeys for 25 years in nutrition, reproduction, and diabetes; and our results are similar to those of other people with other species. Through partnerships, although we cannot supply you with primates, we can certainly do some of your research, which will also indirectly alleviate the pressure on primate populations in user countries.

DR. TARDIF (Suzette Tardif, Southwest National Primate Research Center): I would like to reiterate a point Dr. Morton made about taking this message to the investigators and educating the investigators. I would like to put forward the efforts of the primate centers regarding marmosets as an example of a way to do this.

Colleagues from New England and Wisconsin and I have started to put together some educational efforts. We are going to present a symposium at the AALAS meeting, for example, on marmoset husbandry and management. We are planning a national meeting organized around the use of the European marmoset research group as a model to try to pull together investigators from NIH-supported projects and from pharmaceutical firms, along with people who can supply both marmosets and information about marmosets, to get the word out and to educate people.

We will let you know how it progresses. I think a great deal of effort for individual species must originate from groups like this, composed of investigators who can put together such efforts.

DR. MCGREAL (Shirley McGreal, International Primate Protection League): There is no exact definition of bioterrorism research. Because I think many people might take a favorable position on medical use, I think they also may be very concerned with the ethical implications of holding monkeys as players in the human dramas we create through our human

misconduct. I notice the increase in demand. What percentage of the demand increases is attributable to AIDS, and what percentage or how many monkeys are going to be involved in bioterrorism research?

DR. MORTON: I am not certain that I understood your question, but I think you are basically asking whether we should use monkeys for the human problem we created and how they will be involved? Fortunately for us and perhaps unfortunately for the monkeys, they are the exact model that will be needed to evaluate the kinds of infectious disease that will be used as potential bioterroristic approaches. Some of these agents are already in use, and some are well known. I think smallpox is one such agent. Much of the existing AIDS vaccine work follows along those lines because of the approach. Some of the theoretical vaccines being tested are based on a type of smallpox approach. Much of that work has already been done. Anthrax obviously is one well-publicized agent, and work on the anthrax vaccine has been going on for some time. I believe that situation will just accelerate. I do not know to what degree and I do not think any of these programs have a significant number of primate uses, but I think we all expect to see other kinds of programs that deal with the "Big 5" potential bioterror pathogenic organisms. Obviously, NIAID has huge funding that has been or is being projected to prepare the nation for bioterror threats, and some of that funding will be applied to primate use.

DR. KLEIN: Nonhuman primates are required as part of the proposals for at least two of the bioterrorism agents, anthrax and smallpox, to prove efficacy of antibody development and safety. It appears that industry might be considering non-nonhuman primate species to get preliminary answers to the antibody generation question and parts of the answer to the safety question. Answering some of these questions is more of a national defense priority than other things at this point in time.

DR. MWENDA (Jason Mwenda, Institute of Primate Research, Kenya): I certainly support the idea of the international primate plan as a way of enhancing international collaboration. In Kenya, we have facilities that fulfill the international standards, and we have developed private models for looking at different aspects of infectious diseases, reproductive health, virology, and primate conservation. I believe there is an opportunity to make scientists aware that some of these models are available. Perhaps we could develop a list that highlights some of these models that have been developed over the years and look at their advantages or the advantages and disadvantages of some of these models to actually make people aware that these models are available. I think that would be a good way to start and would basically enhance the collaboration.

My other point is about the cost of private research. We are saying that primates are very expensive; however, with the models that are currently well established, I think we could also reduce the cost of private

research by conducting the research in those resource countries where some of these models are well characterized. That approach could reduce the cost.

DR. ERVIN: I want to expand on Dr. Morton's response to Dr. McGreal's question and simply point out that one of the silver linings of the bioterrorism research demand is that, fundamentally, we are turning attention to the development of vaccines that were formerly relevant only to the third world, or people who could not pay enough to enter as corporations. Now suddenly the first world is threatened, and we are paying attention. The vaccines are not just for evil plots against each other, but they in fact spill over into providing research and protection, we hope, for things like ebola and plague, which are not first world problems. I think there is probably a true benefit there.

DR. GALLAND (Gale Galland, CDC): I look around the room and am awed to see people for whom I have much respect and from whom I have learned so much from over the years. I have only one comment because I know that many people here have the same kind of problem I have: how to convince people in management that they must plan for the future.

Everyone in this room agrees with everything that has been said about conservation and setting up breeding colonies and things like that. I am talking about convincing people—the users, the end-users, and the researchers—but mostly the management people who live from year to year without knowing how much money they will have to spend or what the disease will be next year. It can be a very difficult thing to persuade them to think outside the box and to put money into breeding situations because their first question is, "How many animals can I get out of it this year?" or "How many can I get next year?" It can be very difficult.

I am very interested in any suggestions that might come out of a national primate plan. They look at what they are doing now, what diseases are killing people now, what outbreaks they have now, what they need now to run this study, and so forth. That part of my job is very difficult.

DR. VANDEBERG: I agree that it is a difficult part of the jobs of many of us. I think that an international primate plan should include convincing arguments to support the need to look to the longer term future rather than simply to the immediate needs of this year or maybe next year—not only to project the needs, but also to make the case very clearly that those needs must be thought of many years in advance, rather than when they actually arise.

We have talked about an international primate plan. I want to suggest three other areas that I think are unresolved and perhaps do not fit into the concept of an international primate plan but that might merit a very

detailed study by panels of experts. The first area is the transportation issue, which has been discussed repeatedly. It is very complex and is a moving target, shifting all the time. Might it not be worthwhile to put some real time and effort into defining precisely what the problems are and how they have arisen and into making recommendations in relation to how they might be solved?

Second, we have heard about the need for microbiological standardization and characterization. We all agree it is important, but do we all know what it means? I certainly do not. It is not my field. But do all of the people in that field even agree on the appropriate level of microbiological characterization of our nonhuman primate resources and how we could achieve standardization? Perhaps we need a group of people to invest time and effort into thinking those problems through and making some international recommendations.

The third area that surfaced only briefly (perhaps in a question Dr. Hearn asked yesterday) is the question of genetic standardization. Twenty years ago, there was very little genetic research being done with nonhuman primates—very little genetic monitoring and very little genetic management. We hear increasingly about both the research and management aspects, and Dr. Hearn asked if there are any minimal standards for genetic management of nonhuman primate resources. I recall answering that "it depends on what kind of resource and what the purpose was." Clearly if the answer is not clear in my mind, as one who has worked in genetics all of these years, it is perhaps an issue that needs to be discussed thoughtfully by a more diverse group of geneticists as well as users of animals who might be able to reach a consensus on minimal standards for different types of research and resource colonies. I mention those ideas to the group as possible future initiatives that could arise from this workshop.

DR. ZURLO (Joanne Zurlo, Director of ILAR): At the risk of sounding self-serving on the part of ILAR, I do think we can offer the forum for accomplishing many of the goals that might be set as a result of this meeting. As a first example, Nelson Garnett mentioned yesterday that OLAW is definitely interested in funding a study on transportation. At ILAR Council, the International Committee has discussed the necessity of doing such a study, and so I know that OLAW will contribute some of the money. I think we can probably find resources to fund the remainder of the study, but that matter is still ongoing.

Second, the issue of microbiology and genetics, and even the issue of an international nonhuman primate plan, might be topics for projects that ILAR can orchestrate probably better than many other institutions in the country in the sense that we do have contacts and working relationships with the Federation of European Laboratory Animal Science Associations

(FELASA), with the European Union, with the Pan American Health Organization (PAHO), and through the primate research centers to some of the Asian and African countries. The resources that allowed us to arrange this meeting could also allow us to serve as the foundation for working on an international plan. So, I offer that possibility, and I offer our services. I am sure that the representatives from ILAR Council who are here will underscore that offer and echo that sentiment.

DR. VANDEBERG: Thank you for offering that opportunity to all of us.

DR. SEIER: Maybe a small solution or part of the solution to the problem is not to transport the primates but to transport the users, unless airlines will also refuse to transport them in the near future. I think you will find that in facilities that meet your requirements for a good standard, the work will be much cheaper than in many user countries, and there will be plenty of margin/surplus for transporting the user.

DR. HEARN: It occurred to me after hearing the comment from Dr. Galland that a number of these issues are articulated clearly in the 1978 plan. I think we could review the plan to see that it has some very clear and powerful language.

To return to Dr. Robinson's point, it seemed to be a surprise to some that suddenly there is a high-level committee looking at the future needs of primates in a multiagency context. Unfortunately, many of the people (and I am a cynic) who sit on these national and international level committees probably could not tell a monkey from a mall rat, but it is an opportunity for leadership. The important point is that the leadership should come from the community that has clear information. I suggest that information should be provided on primate needs in terms of numbers and endpoints of that, on the needs for primate research that furthers our knowledge, and on slightly different, more currently urgent needs on bioterror. These needs are not the same. We need clarity and we need to address all of them.

I think we need clarity also in this interaction among ourselves, as scientists, biologists, biomedical researchers, and conservationists. I am always impressed with Dr. Mittermeier's wonderful vision and the things he is doing; I am also very impressed in a meeting like this to learn that so many things are being done in biomedicine. But let us not kid ourselves that we are all "fuzzy conservationists." We all need to take note of conservation and be conservationists in the broader sense, but let us have clarity in that the endpoints and the objectives in biomedical research and in conservation biology need to be kept fairly straightforward and simple because the communities are not all the same. Mixing them up may not always be to the advantage of either; however, I suggest that we do need to give intellectual and practical application to the overlap of those two

objectives where we can definitely synergize. The new resources in genetics can be applied in the field, as can most of the upcoming technologies for noninvasives and the possibilities of preventing disease in primates in the field with new delivery systems. These applications are all very valid, so I think we can view them as part of an international collaborative program.

Finally, I believe that the process the ILAR Council has developed over the years in delivering best practice across animal sciences is a system that works extremely well for consultation and delivery of a report. To follow that process may be the best way forward here in a relatively independent way to acknowledge and integrate the different bodies from NIH, from industry, and from the international community in delivering quickly a clear and fairly simple international primate plan.

DR. ROBERTS: I would like to make one comment on your references to the microbiological and genetic characterization and as one of the members of the SPF group that was supported by NCRR through OAR. Some of these issues might be a framework for an initial consideration. Dr. Mansfield may want to add in a comment, or not, as he is a leader of that group. That group will be larger with the new RFA soliciting applications that deal not only with rhesus but also with other species of macaques. Certainly we have a long way to go before we come close to the standards established in rodent biology with respect to nomenclature, disease characterization, and genetic management. However, those are goals for which we have those examples.

As Dr. Tardif mentioned about thinking in terms of the long term, a rodent may require 20 generations to achieve an inbred strain. With primates, we should think 10 and 20 years into the future about what we are going to need and will hopefully have for our populations then. Certainly things will change in the meantime, but I believe the old saying is appropriate: "It's not the plan that's important, it's the planning process that's important."

DR. VANDEBERG: To conclude, I would like to offer our appreciation to several parties. First, to ILAR for having perceived the need for this meeting, having organized the conference, and having done all of the hard work to make it possible. Second, to NCRR, as the principal sponsor of this workshop, without which we could not have had it. And finally, to all of the speakers who did a superb job and pitched their talks exactly where we hoped they would. They, together with the audience, are really responsible for this stimulating meeting. I thank all of you and our panel for their comments this morning.

Conference Summary

Conference Summary

William R. Morton, VMD

The task of summarizing this workshop has been very difficult; however, I have had the help of a number of individuals, particularly Drs. Abee and Klein. Together we have culled several relevant points, which I will describe.

In opening the session, Dr. VandeBerg established two important points about nonhuman primates: the number used in research is decreasing; and they have become less and less accessible over the last several years. That combination of factors can and will have an impact on our ability to do biomedical research on human health problems. We need to work diligently to communicate the value of human health research to the people in the areas or countries of origin and resource providers here. In addition, as a spin-off, animal health improvements and progress are often the result of our human health research efforts. We can avoid *future* shortfalls of nonhuman primate resources by making a long-term investment *now* in the resources and the infrastructure needed to provide them for biomedical research. We have heard that theme repeatedly throughout the last 2½ days.

There is a need for a continuing and increased investment in infrastructure for nonhuman primate-based research. As Dr. Hearn emphasized in the keynote address, this is a unique time in science, when the

Director, Washington National Primate Research Center, Seattle, WA

basic sciences, the applied sciences, and human health problems are coming together at an unusually rapid rate. We hope that the efforts we and others in the research field are making will translate rapidly into human health applications. Some of the current research efforts with nonhuman primates, particularly in the field of AIDS, offer great potential in the development of antivirals and, hopefully, effective vaccines. In the future, some of the strategic biotechnology areas of research that will loom larger are AIDS, stem cell research, gene therapy, aging, and learning and behavior. These fields may require the use of nonhuman primates to assure progress in human medicine.

However, to achieve the necessary balance between environmental needs, conservation needs, and the provision of resources, we need alternatives to the use of the rhesus monkey (*Macaca mulatta*). Dr. VandeBerg urged NCRR (National Center for Research Resources), ILAR (Institute for Laboratory Animal Research), and other such agencies to take a combined leadership role in providing the resources to allow us to move ahead in a thoughtful way in the use of the nonhuman primate. He outlined prospective actions and recommended that we build on the resources to fund the national primate centers and other nonhuman primate facilities to conduct state-of-the-art research. He described coalitions between institutions such as the National Academy of Sciences (specifically ILAR), NIH (National Institutes of Health), USDA (US Department of Agriculture), and private industry that would result in the development of even better international collaborations. There must be a mutual outreach between the national and the international efforts to provide not only what *we* need, but also what *they* need. We must work toward conservational funding as well as resource sharing.

Several speakers expressed increasing concern in regard to bioterrorism and the research surrounding that concern, including vaccine development. Vaccines for use throughout the world are the ultimate and tremendously valuable product of these efforts.

Our international guests, who have come from virtually every corner of the globe, summarized their needs and problems in an effort to foster a more positive collaborative approach between the countries of use and the countries of origin of these nonhuman primate species. A critical question raised throughout the presentation was, "How can we better interact and perhaps use some of the existing programs as prototypical models while developing even better methods for the future?"

We all share in the concern for the destruction of these nonhuman primates' habitat, which has forced many of these and other species into a pest-like status and has contributed to their destruction. Our international colleagues clarified their particular community needs as well as their cultural and philosophical differences. They described their concerns regard-

ing the inadequacies of the existing infrastructure, regulatory burden, and transportation issues. These issues are both current and future concerns, which we intend to address in future meetings.

Dr. Robinson provided an overview of domestic nonhuman primate resources from the NIH perspective. He described the increase in nonhuman primate use, plans for the future, and resource concerns. There will be a subsequent meeting to elaborate on those concerns.

Dr. Beattie presented a unique snapshot of the private sector/private industry use of the nonhuman primate. The information was very revealing in terms of that industry's direction and the needs we must fulfill.

At the close of the first day, we had a very detailed presentation of a long-awaited, newly published update of the National Academies publication *Nutritional Requirements of Nonhuman Primates*. It is greatly welcome and should be utilized in the field.

At the beginning of the second day, Dr. VandeBerg strongly supported the use of genetics and genetic tools in research that utilizes the nonhuman primate for some of the outstanding examples he gave as significant causes of human morbidity and mortality. He described how research could improve and be of higher quality through the use of increased technology and improved technology of genetics. The value of the nonhuman primate model could be expanded by using these tools to address disease issues.

Dr. Williams-Blangero strongly advocated better management of nonhuman primate colonies through the use of genetics. She urged greater attention to pedigree and to developing good pedigrees by focusing more on data collection and records of all types. She presented breeding strategies that could be used in colony management based on these genetic considerations. Southwest Foundation has developed software that is available to assist nonhuman primate colony managers.

Dr. Friedrich presented a detailed description of the prototypical antiviral infection in the nonhuman primate, from exposure and infection, to the initiation of immune mechanisms, to the assumption of control, and ultimately to disease. He pointed to the role the genotype of a particular nonhuman primate can play in helping to control the infection. He presented the example of the Mamu-A*01 MHC class 1 allele, which has highlighted the genetic interaction with viral infection and AIDS in the rhesus monkey.

Mamu-A*01 is perhaps the most important single factor that has led to this meeting. The discoveries are continuing, and other alleles have already been identified that further control MHC class 1 and class 2 alleles in AIDS pathogenesis, vaccine production, and research. The following questions arise: Where do we go; how much do we design these animals for research; and what is the cost of that design? By combining those

questions with Dr. Mansfield's talk (described below), it is possible to visualize the ultra-genetically designed animal with the specific pathogen-free animal—with costs escalating to exceed those of the chimpanzee!

Mr. DeMarcus presented an overview of importation, quarantine, and transport concerns from the perspective of the CDC (Centers for Disease Control and Prevention) (see below).

Dr. Marthas focused on aspects of studying one species exclusively and suggested that we consider looking at the same species from different origins, that is Chinese-origin versus Indian-origin rhesus monkeys. Comparison of SIV (simian immunodeficiency virus) pathogenesis in those two particular types of nonhuman primates has shown that there are no significant differences. There has been more heterogeneity in the Chinese-origin rhesus monkey in considering a variety of factors, but basically the outcomes have been the same. Both have shown they have the ability to be infected. Both have shown progression to the disease, and the variability in both sets has been relatively similar. Those findings have emphasized the fact that genetic profiling of nonhuman primates, as much as possible before the design of experiments, could have a positive impact on the outcome of those experiments.

The fact that there is very little in the literature that scientifically compares differences between species is another important consideration that has emerged from this meeting. More scientific studies should be devoted to comparative studies between nonhuman primate species and making those species available for various types of research. Drs. Friedrich and Marthas' presentations addressed this matter in terms of proposed action.

In the Microbiology session, contributors included Drs. Klein, Baskin, DeMarcus, Motzel, and Mansfield. Dr. Baskin presented an overview of specific micro-organisms (i.e., pathogenic organisms) in the nonhuman primate and associated concerns, including the more common but significant colony health concerns such as chronic colitis. He outlined the need to define the nonhuman primate (particularly the macaque) microbiologically.

One of the planned goals of this conference was to address microbiological standardization of various assays, reagents, and methodologies being used for testing in these animals, particularly in the macaque. These issues must be addressed in more depth and should be the subject of discussion in future conferences. Dr. Baskin made the appeal, as have others, for increased funding for infrastructure to provide better laboratories, better facilities, better necropsy facilities, better capability of microbiological assessment, and ample and adequate training.

We learned from Mr. DeMarcus' presentation that in terms of species, *Macaca fascicularis* (cynomologus) accounted for more than 80% of all non-

human primates imported to the US during the fiscal year 2001. That number represented a huge portion of nonhuman primates coming into the United States, with the rhesus accounting for the second greatest number, at 13%. All other primate species were almost negligible in terms of numbers used.

Importantly, the mortality figures for importing quarantine were exceedingly low, at 0.7%. That low figure represents a major change over the years. I can remember when the percentage was much higher, and I think that change is very positive. We may have blundered in many other areas, but this change is a great improvement over the past. Mr. DeMarcus also emphasized animal transportation issues and the emerging needs regarding quarantine issues and limited airlines.

Dr. Motzel presented a comparative study in which she confirmed that tuberculosis in the nonhuman primate is not a disease of the past but will always be a threat. No single method of testing primates for the disease is infallible, and it is imperative to use multiple tests and a multiple means of diagnosis. Dr. Motzel compared the African greens, the cynomolgous, and rhesus monkeys and showed us once again that the African greens are extremely sensitive to this infection, as are the rhesus monkeys for the most part. The cynomolgous monkeys, for whatever reasons, are much more tolerant of infection by *M. tuberculosis*.

Unfortunately, testing every 6 months may not be enough because as Dr. Motzel pointed out, it is already too late when the first sign (usually a cough) becomes evident. At that point, other animals have already been exposed. She emphasized the need to constantly review the testing. She pointed to radiological methods that were effective in detecting specific, early pulmonary forms.

Dr. Mansfield's presentation focused on one example of how to derive and create an SPF (specific pathogen-free) colony. He described the need to survey for an array of nine viruses. In spite of the great difference between the rodent and the nonhuman-primate fields in terms of genetic and microbiological definition, we appear to be approaching a better genetically and microbiologically defined macaque for research.

Many individuals discussed transportation issues for nonhuman primates in terms of regulatory issues and transportation impeding the supply of reliable animal models. We repeatedly focused on airline problems in this meeting, which surely should be one subject of a future meeting.

Many overviews were presented from several different perspectives: Dr. Garnett from OLAW (Office of Laboratory Animal Welfare); Dr. DePoyster from USDA; Dr. Kreger from the US Department of Fish and Wildlife. Drs. Rapley and Hsu focused on conservation efforts, and Dr. Hsu described Chinese resources and which airlines transport nonhuman primates from China. This topic is very practical because the

Chinese airlines serve not only China but also the entire Southeast Asian resource needs (e.g., both China and China Southern Air serve Indonesia).

The speakers identified the following agencies as the major regulatory "drivers" of transportation: IATA (International Air Transport Association); CITES (Convention on International Trade in Endangered Species of Wild Flora and Fauna) concerns; USDA; and US Fish and Wildlife. All of these agencies would benefit from a coordinated approach as well as increased understanding of the issues surrounding transportation of nonhuman primates. Often we can satisfy one regulatory agency but not satisfy another. We need to identify and resolve overlapping and conflicting requirements to develop a nonhuman-primate plan that is truly international in scope.

As a transportation issue, the limited number of airlines came to the fore again and again, as well as the delays in transport, which are caused by many factors. Again, the problems are regulatory permits, the routing of air traffic, and the various reasons that traffic must go one place or another.

In the final panel discussion, we discussed strategies to integrate all of our mutual concerns as we utilize the nonhuman primate. Participants called for the *rapid* development of an international primate plan that is needed *now*. ILAR appears to be the best suited organization to facilitate this endeavor. ILAR is nongovernmental, yet its Council members have access to scientific and management expertise related to US research and a representative knowledge of the other countries that should play a central role in developing a successful international plan. As Dr. Zurlo stated, plans are already under way to develop a transportation workshop or study. Hopefully, we will then proceed with the plan for microbiologically standardizing specific nonhuman primates (particularly the macaque and perhaps baboon) and for more genetic standardization.

I would like to echo Dr. VandeBerg in thanking both ILAR and NCRR for hosting this workshop. Thanks are due Drs. Abee and Klein for their assistance with this summary, and all of the ILAR staff for a very beneficial meeting.

DR. ZURLO (Joanne Zurlo, Director of ILAR): I have nothing to add to what has been said already; however, I do want to offer my heartfelt thanks to the speakers at this meeting. The presentations were beyond expectation, and we owe the success of the meeting to the speakers' preparation and participation. A very, very special thank you is due the Program Committee, who made the meeting possible through their inspiration and ideas for topic development. Thanks are also due ILAR Council because the work of the Institute functions through its Council, a very strong and important group with which I am privileged to work. Additional thanks are due the audience, for tremendous participation in the

meeting. From a personal perspective, this meeting has been a culturally rich opportunity for me to meet many new people and share experiences. I am very happy that all of you could be here, and I wish everyone a safe trip home.

Appendix A

International Perspectives: The Future of Nonhuman Primate Resources Program

PROGRAM

National Academy of Sciences Auditorium
April 17-19, 2002

Wednesday, April 17, 2003

8:30 am	***CONTINENTAL BREAKFAST—Great Hall***	
9:00 am	*Welcome and Introduction*	**Joanne Zurlo, PhD** Director, ILAR **John VandeBerg, PhD** Southwest Foundation for Biomedical Research
9:15 am	*Primate Priorities—International Perspectives*	**John P. Hearn** Australian National University
10:00 am	***BREAK***	
Session 1:	***Conservation and Supply; short presentations and panel discussion by representatives from major countries of origin of NHP***	**Christian Abee, *Chair*, DVM** University of South Alabama

10:30 am	*Overview of Biomedical and Conservation Research Using Kenyan Nonhuman Primates*	**Jason Mwenda** Institute of Primate Research, Kenya
10:45 am	*Supply and Use of Nonhuman Primates in Biomedical Research: A South African Perspective*	**Jürgen Seier** Medical Research Council, South Africa
11:00 am	*Title TBA*	**Joko Imung Pamungkas** Primate Research Center, Indonesia
11:15 am	*Use of Nonhuman Primates in Biomedical Research in India: Current Status and Future Prospects*	**A. Jagannadha Rao** Indian Institute of Science
11:30 am	*Initiatives of Primate Captive Breeding in Nepal*	**Mukesh Kumar Chalise** Natural History Society of Nepal
11:45 am	*Chinese Primate Status and Captive Breeding of Primates for Biomedical Research in China*	**Zhiyong Fan** Endangered Species of Wild Fauna & Flora Import & Export Mgmt. Office of China
12:00 noon	***LUNCH—Great Hall***	
1:00 pm	*The Breeding of Naturally Occurring B-Virus Free Cynomolgus Monkeys on the Island of Mauritius*	**Mary Ann Stanley** Bioculture, Ltd., Mauritius
1:15 pm	*Primates for Research in Bolivia*	**Mario J. Baudoin** Ministry of Sustainable Development and Planning
1:30 pm	*The St. Kitts Vervet: An International Resource*	**Frank Ervin** McGill University, Canada
1:45 pm	*PANEL DISCUSSION*	

Session 2:	***Conservation and Supply; short presentations and panel discussion by representatives of other primate producing countries***	**John Vandenbergh, *Chair*, PhD** North Carolina State University
2:15 pm	*Nonhuman Primates in Preclinical Research—the European Situation*	**Gerhard Hunsmann** German Primate Centre
2:30 pm	*Providing with Laboratory Primates Researchers and Vaccine Producers in Russian Federation*	**Boris Lapin** Institute of Medical Primatology, Russia
2:45 pm	***BREAK***	
3:10 pm	*Nonhuman Primate Resource Needs: A Moving Target*	**Jerry Robinson** National Center for Research Resources, NIH **Greg Beattie** Sierra Biomedical, Charles River Labs
3:50 pm	*Center for Conservation and Reproduction of Primates—Peruvian Primatology Project*	**Enrique Montoya** Peruvian Primatology Project
4:10 pm	*PANEL DISCUSSION*	
Session 3:	***Nutrient Requirements of Nonhuman Primates***	**Committee on Animal Nutrition, Board on Agriculture and Natural Resources, NRC**
4:40 pm	*Newly Published Update of Report*	**Duane E. Ullrey, *Chair*** Michigan State University **Lynne M. Ausman** Tufts University **Lawrence L. Rudel** Wake Forest University School of Medicine **Sherry M. Lewis** Bionetics Corporation

Thursday, April 18, 2003

8:30 am	***CONTINENTAL BREAKFAST—Great Hall***	
Session 4:	***Genetics***	**John VandeBerg, PhD, *Chair*** Southwest Foundation for Biomedical Research
9:00 am	*Nonhuman Primates in Genetic Research of Primates—Peruvian Primatology Project on Common Diseases*	**John VandeBerg, PhD**
9:30 am	*Genetic Considerations in Captive Management and Research with Nonhuman Primates*	**Sarah Williams-Blangero** Southwest Foundation for Biomedical Research
10:00 am	*Association of Particular MHC Class I Alleles with Control of AIDS Virus Infection*	**Thomas Friedrich** Wisconsin Regional Primate Research Center
10:30 am	***BREAK***	
11:00 am	*Chinese-origin Rhesus and Cynomolgus Macaques for AIDS-Related Research*	**Marta Marthas** California Regional Primate Research Center
11:30 am	*PANEL DISCUSSION*	
12:00 noon	***LUNCH—Great Hall***	
Session 5:	***Microbiology***	**Hilton Klein, *Chair*, VMD** Merck Research Laboratories
1:00 pm	*Microbiologic Problems in Nonhuman Primates Used in Research*	**Gary Baskin** Tulane Regional Primate Research Center
1:20 pm	*United States Nonhuman Primate Import Quarantine*	**Tom DeMarcus** Centers for Disease Control and Prevention
1:40 pm	*Diagnosis of Tuberculosis in Nonhuman Primates*	**Sherri Motzel** Merck Research Laboratories

2:00 pm	*Specific Pathogen Free Rhesus Macaques*	**Keith Mansfield** New England Regional Primate Research Center
2:20 pm	*PANEL DISCUSSION*	
2:50 pm	***BREAK***	
Session 6: *Transportation*		**William Morton, VMD, *Chair*** Washington Regional Primate Research Center
3:15 pm	*An OLAW Perspective*	**Nelson Garnett** Office for Laboratory Animal Welfare, NIH
3:30 pm	*Non-Human Primate Transportation Regulations*	**Jerry DePoyster** APHIS/USDA
3:45 pm	*International Transportation of Nonhuman Primates: U.S. Fish & Wildlife Service Perspective*	**Michael Kreger** U.S. Department of the Interior
4:00 pm	*Primates—Conservation: Status in the Wild and Transportation*	**William A. Rapley** Toronto Zoo Canada
4:15 pm	*Chinese Macaques—East Meets West*	**C. K. Hsu** Shared Enterprises, Inc.
4:30 pm	*PANEL DISCUSSION*	
5:30 pm	***Reception and Banquet with speaker (ticket required)***	**Russell A. Mittermeier** Conservation International

Friday, April 19, 2003

8:30 am	***CONTINENTAL BREAKFAST—Great Hall***	
Session 7:	***Unresolved Issues***	**John VandeBerg, *Chair*, PhD**
9:00 am	*Panel with Session Chairs to work with audience in defining issues that need detailed study)*	

10:30 am	***BREAK***	
11:00 am	*Conference Summary*	**William Morton, VMD**
12:00 noon	*Workshop Adjourns*	

Appendix B

Glossary of Abbreviations

AATA, Air Animal Transportation Association
AIDS, acquired immunodeficiency syndrome

BCM, Bioculture Mauritius Ltd.

CAMP, Conservation Assessment and Management Plan, Indonesia
CBSG, Conservation Breeding Specialist Group
CCIUCN, Canadian Committee for the World Conservation Union
CDC, Centers for Disease Control and Prevention
CITES, Convention on International Trade in Endangered Species of Wild Flora and Fauna
CMV, cytomegalovirus
CPE, cytopathic effect
CRCP, Center for Conservation of Primates of Iquitos
CTL, cytotoxic T-lymphocyte

DNPWC, Department of National Parks and Wildlife Conservation, Nepal

ELISA, enzyme-linked immunosorbent assay
EMCV, encephalomyocarditis virus
ESA, Endangered Species Act of 1973
ESR, erythrosedimentation rate
EU, European Union

FY, fiscal year

HIV, human immunodeficiency virus

IEPaT AMS, Sukhumi Institute of Experimental Pathology and Therapy of the USSR Academy of Medical Sciences
ILAR, Institute for Laboratory Animal Research
IMP RAMS, Institute of Medical Primatology, Russian Academy of Medical Sciences
INF-γ, interferon-gamma
IPR, Institute of Primate Research, Nairobi, Kenya
IUNC, World Conservation Congress
IVAG, intravaginal
IVITA, Veterinary Institute of Tropical and High Altitude Research

LAR, Live Animals Regulations of the International Air Transport Association
LCV, lymphocryptovirus

MHC, major histocompatibility complex
MOU, memorandum of understanding
MOT, mammalian old tuberculin

NAHSON, National History Society of Nepal
NAS, National Academy of Sciences
NCRR, National Center for Research Resources
NENPRC, New England National Primate Research Center
NHP, nonhuman primate
NIH, National Institutes of Health
NPRC, Nepal Primate Research Center
NPRCC, National Primate Research Center Program

PAHO, Pan American Health Organization
PCBF, primate captive breeding farm
PCR, polymerase chain reaction
PI, postinfection
PPD, purified protein derivative
PPE, personal protective equipment
PPP, Peruvian Primatology Project
PVHA, population viability analysis

RRV, rhesus rhadinovirus

SFV, simian foamy virus
SHFV, simian hemorrhagic fever
SIV, simian immunodeficiency virus
SPF, specific pathogen free

SRV, simian retrovirus
SRV-D, simian retrovirus type D
STLV, simian T-lymphotropic virus
STLV-1, simian T-lymphotropic virus type 1
SVV, simian varicella virus

TB, tuberculosis
TRPNR, Tana River Primate Reserve, Kenya
TU, Tribhuvan University, Nepal

UNMSM, School of Veterinary Medicine of the San Marcos National University
USDA, US Department of Agriculture
USFWS, US Fish and Wildlife Service

WaNPRC, University of Washington Nepal Primate Research Center
WHO, World Health Organization
WTO, World Trade Organization

Appendix C

Committee Bios
International Perspectives: The Future of Nonhuman Primate Resources

PROGRAM COMMITTEE

John L. VandeBerg (*Chair*) is Scientific Director of the Southwest Foundation for Biomedical Research, where he holds the Corwin D. Denney Distinguished Scientist Chair. He also is a Professor of Cellular and Structural Biology and of Pathology at the University of Texas Health Science Center at San Antonio. He received B.S. degree from the University of Wisconsin-Madison, and then traveled as a Fulbright Scholar to Australia where he received B.Sc.Hons. degree from La Trobe University in Melbourne, and a Ph.D. degree from Macquarie University in Sydney. All of his degrees were in genetics. Dr. VandeBerg did his postdoctoral training in genetics with Prof. William H. Stone at the University of Wisconsin-Madison and then spent a year at Wisconsin as an Assistant Scientist jointly appointed in the Laboratory of Genetics and at the Wisconsin Regional Primate Research Center. Dr. VandeBerg moved to the Southwest Foundation in 1980, founded its Department of Genetics in 1982, and became Scientific Director in 1994.

Christian R. Abee, D.V.M., M.S. is The Charles M. Baugh Professor and Chair of the Department of Comparative Medicine at the University of South Alabama College of Medicine in Mobile, Alabama. Dr. Abee is a former member of the ILAR Council and the National Center for Research

Resources (NCRR) Comparative Medicine Review Committee. He is director of the Squirrel Monkey Breeding and Research Resource (SMBRR), a research center that specializes in providing resources and conducting research using squirrel monkeys (Saimiri spp.). This center is the only NCRR/NIH supported center that specializes in Neotropical primates.

Janet C. Gonder was Vice President of the Center for Assessment of Safety and Efficacy, Baxter Healthcare Corporation. Her expertise is in laboratory animal medicine, knowledge of international activities and participation in the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Hilton J. Klein, VMD, M.S., is Senior Director for Comparative Medicine, Merck Research Laboratories, and Adjunct Assistant Professor, Department of Laboratory Animal Resources, University of Pennsylvania. His background is in laboratory animal medicine. His research interests are in laboratory animal science, particularly in the field of laboratory animal infectious disease and surgical production of animal models. He has been a consultant to the Pan American Health Organization as Merck's representative on nonhuman primate conservation.

William R. Morton, VMD, is Director, Regional Primate Research Center, University of Washington and Director of AIDS Research at the Regional Primate Research Center. He has an extensive background in primatology. His research interests are retrovirology and has published extensively on SIV variants and vaccine development. He is a well-known primatologist and has been an officer of the Association of Primate Veterinarians.

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