Macaca mulatta : management of a laboratory breeding colony / David Allen Valerio [and others].

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MACACA MULATTA:
MANAGEMENT OF A LABORATORY BREEDING COLONY

This book is concerned with the establishment of simian colonies for laboratory breeding. It also provides information on the geographic range, classification, taxonomy, and statistics of the free-ranging monkey. Topics treated in length are building and cage designs, husbandry, nutrition, physiology and reproduction, pregnancy, care of newborn and juvenile monkeys, and diseases (their prevention and treatment). Key references to pathological literature are given.

A practical guide on the **Macaca mulatta,** the book will be of vital importance to laboratory veterinarians, scientists and experimentalists who use large number of monkeys, and animal supervisors and their aides.

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Macaca mulatta

Management of a Laboratory Breeding Colony

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PREFACE

In February 1962, Bionetics Research Laboratories, Inc. was awarded a contract under the Special Virus Leukemia Program, of the National Cancer Institute, to produce and maintain monkeys under rigidly controlled conditions for long-term studies on leukemia. Major objectives of this program are the determination of whether viruses similar to those known to induce leukemia and lymphoma in laboratory and domestic animals are also involved in the induction of these diseases in man and, when such viruses are found, the development of effective vaccines or other means for the prevention of these diseases. If human leukemia can be transmitted to a simian, the experimental disease will provide a biological test system that will help to elucidate pathogenesis and can then be used to find ways of combating the human disease(s).

Institution of our program involved many problems. Monkeys are not readily available in the quantities in which other laboratory animals can easily be obtained. Not only is a plentiful supply of healthy baby monkeys needed for experimentation, but the animals must be housed in a facility where a careful watch may be kept over their health and well-being and where their condition can be closely monitored for periods of up to ten years. In addition, the facility has to provide adequate safeguards for personnel against the potentially infectious nature of the experimental materials (i.e., neoplastic tissues from human sources).

Since the award of the contract, we have accumulated a fund of knowledge in the establishment and maintenance of a large simian colony geared to production of infants. Much of the knowledge has been gained by the sometimes painful process of trial and error. In recognition of this, the National Cancer Institute asked BRL to

share its experience with other laboratories that are venturing into the field of raising experimental monkeys.

The initial contract specifications emphasized the inoculation of experimental materials into baby monkeys as soon as possible after birth—hopefully within 12 hours. This, obviously, required that the birth occur in the laboratory. In turn, this necessitated acquisition of pregnant females; later, when the laboratory had adult males and females on hand, inhouse breeding filled most of the demand. One way or another, the babies were made available. More than 3500 babies have been born—over 600 in the past year. The oldest infant now alive was the eighth—born in April, 1962. At present, a breeding colony of about 900 produces some 90% of the babies.

To conduct its leukemia research program, BRL built a modern facility at Kensington, Maryland, a Washington suburban location that precluded outdoor housing and demanded minimum land usage. As an animal housing facility, the building is unusual because of its compact housing system and multistory design.

This guide describes the facilities and procedures in operation at the time of writing. Their primary virtue is that they work. Other institutions may not be established in the same manner because research missions differ. However, the basic experiences reported here, and the specific component activities within our operations, should be applicable to the use of the simian employed in a variety of medical research.

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Robert Lawrence Miller
James Robert Maitland Innes
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Arthur Joseph Pallotta
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March, 1969

Publisher's Note: Drs. Valerio, Innes, and Pallotta, and Mr. Guttmacher are presently affiliated with Bionetics Research Laboratories, Inc., Bethesda, Maryland. Dr. Miller is associated with the Seven Corners Animal Hospital, Falls Church, Virginia, and Dr. Courtney with the National Environmental Health Sciences Center, Research Triangle Park, North Carolina.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical and financial support of the National Cancer Institute, National Institutes of Health, under Contract Programs PH 43-62-412 and 43-67-661, without which this guide could never have been written. Particular thanks are given for the able and understanding direction of Dr. Frank J. Rauscher, Jr., Project Officer of this program, Drs. W. Ray Bryan and John B. Moloney, and other members of the staff of the National Cancer Institute.

The material contained in this guide is the result of hard work by every member – past and present – of our "Simian Research Center." Their diligence and creativity, which we appreciate, resulted in the great success of our project.

Our gratitude is expressed to several of our laboratory workers who contributed notably to certain parts of the guide. All work on hematology was the responsibility of J. Switzer, D.V.M., M.S., who was ably assisted by Mrs. Bonnie Rininger in the voluminous day-to-day examination of blood and bone marrow in all its complexities. The chapter on simian surgery was helped by the considerable experience of J. O. Warfield, M.D. Examinations for parasitic infestations (helminthic and protozoan) were done by Miss Lucy Reardon, M.A. Contributions on diagnostic bacteriology and antibiotic therapy were provided by Kyle H. Sibinovic, Ph.D. Assisting in the difficult task of performing the necropsies and arduous histological studies on hundreds of various species of apes and monkeys were Marion G. Valerio, D.V.M. and Borge M. Ulland, D.V.M. The design of the cages and animal rooms and the scale drawings were the work of Mr. Myron Landon. Our experience in clinical simian medicine dealing with babies and juveniles benefited from the work of Clement Darrow, D.V.M.

We cannot fail to acknowledge the splendid help given to our breeding program by dozens of animal aides and their supervisors; we can only name but a few of the latter – Mr. George Thompson, Mr. Joe Cornett, Mrs. Ileen Maxwell, and Mrs. Marty McGowan. In the compilation of the guide, special thanks must go to additional members of our technical and administrative staff: Mrs. Virginia Durgin, Mr. Ronald Mace, Mr. Thomas Richardson, and Mrs. Ellen Zinkler. Miss Carolyn Kalk gave invaluable help in the preparation of the manuscript.

The authoritative Chapter I, "The Free-Ranging Rhesus Monkey," was written by J. R. Napier, D.Sc. (Director of the Primate Biology Program, Smithsonian Institute, Washington, D. C.) and his wife, Mrs. Prue Napier.

The principles of animal care, as promulgated by the National Society for Medical Research, were strictly observed in all aspects of our simian colony.

We express particular appreciation to *Life* magazine and their photographer, Mr. Fritz Goro, for their gracious permission to use some of the photographs contained in the text.

INTRODUCTION

Before 1940, a few thousand monkeys were imported into the United States for experimental purposes; in 1968 the number was over 120,000. A publication appeared in 1938 protesting the deplorable conditions of importation and transportation and the appalling mortality within days and weeks after arrival.

A high mortality among simians had occurred for decades in zoological gardens throughout the world, but the number of apes and monkeys involved was never very large. Outside such gardens, there were few scientists with any extensive experience in any aspect of simian biology—reproduction, husbandry, medicine, or pathology. Pioneers in the physiology of reproduction, embryology, intrauterine development, and placentation were C. G. Hartman, G. E. Corner, and G. van Wagenen.

The problem of disease and its control in imported simians is perennial and of prime magnitude because diseases carried from natural habitats may be transmitted to other animals in an established colony and to man. The existing literature on simian pathology is limited and, although a book by T. C. Ruch filled a gap, an authoritative, comprehensive text based on wide experience with simian diseases remains to be written.

Until 7 years ago, nearly all simians for experimental use in the United States were imported. In retrospect, the concept of establishing breeding colonies was inevitable. At present, there are seven National Primate Centers in the United States sponsored by the National Institutes of Health, Bethesda, Maryland, but they are not primarily concerned with breeding animals for research. There is no composite publication covering all aspects of simian husbandry that describes the establishment and maintenance of a breeding colony. Most of the existing literature, derived from experience

obtained in zoological gardens or from works on the physiology of reproduction, is incomplete or irrelevant to the complex problems that faced us in establishing a large breeding colony in 1962.

It is probable that in 25-50 years few simians will be used for experimentation except those bred in laboratories. The reasons are obvious. Tracing a parallel to the experimental use of rats and mice (now over 20 million a year), there are incontrovertible advantages in using laboratory-bred monkeys whose health status is well known, rather than using imported animals of uncertain age and condition, which bring with them diseases causing ill health and high mortality often soon after arrival. Although the cost of rearing simians to maturity is high, it is considerably less in the long run than that involved in the purchase of animals from a commercial source and possibly losing many before the start of experimentation or after a costly study is underway. For much of our research, we think in terms of utilizing animals for experimentation for 10 years, at least, or a lifetime (which may be between 20 and 30 years).

Although we use imported *Macaca mulatta* and other simian species for some experiments, our major concern is the development of a breeding colony and the production of healthy newborn simians that will survive to maturity and many years beyond. In our program, newborn monkeys are separated from their mothers at birth and are hand-reared under isolator conditions. Such animals cannot be purchased from a commercial source.

The information in this manual has been garnered by workers in different disciplines. Our aim is the production of a practical guide on *M. mulatta*, mainly to present information to animal researchers or veterinarians who are interested in establishing simian colonies, especially for breeding purposes. Parts of the text, however, should also be of practical value to animal care supervisors who lack specific knowledge and experience in dealing with monkeys.

Although this guide is concerned only with *M. mulatta*, we have had experience with *Cercopithecus aethiops* (African green monkey), *Macaca fascicularis* (*irus*) (cynomolgus monkey), *Macaca radiata* (bonnet monkey), *Macaca nemestrina* (pigtail monkey), *Macaca speciosa* (stumptail monkey), *Saimiri sciureus* (squirrel monkey), *Papio doguera* (baboon), *Saguinus oedipus* and *Callithrix jacchus* (marmosets), and the prosimians, *Galago crassicaudatus* and *Galago senegalensis*.

I THE FREE-RANGING RHESUS MONKEY

Macaca mulatta, the rhesus monkey, has become for many people the archetypal primate. To a layman the word "monkey" recalls an indeterminate creature with a face like a chimpanzee, swinging from the branch of a tree by its prehensile tail, while to a scientist it means the "rhesus." The words "monkey" and "rhesus" have become almost synonymous in biomedical literature to the extent that, in many instances, the species concerned is not even mentioned by name. While this no doubt can be regarded as the ultimate accolade for the rhesus monkey, it can hardly be approved as good scientific practice.

It is worthwhile to review briefly the history of the rhesus monkey in research and the manner in which its name was made.

Macaques are the earliest recorded experimental animals for scientific research but it was not the rhesus monkey that was used by Galen* of Pergamum in 150 A.D. because of its "likeness to man." It was the Barbary macaque (*Macaca sylvanus*) famed as the monkey that lives in ecological harmony with the British on Gibraltar. The first mention of the rhesus monkey in the scientific literature was in a paper by Bland-Sutton in 1886 in the *British Gynaecological Journal*. Bland-Sutton was attempting to discover whether or not menstrual bleeding in the macaque was associated with destruction of the endometrium. The first really impressive contribution of the rhesus monkey to science was made in 1889 when Sherrington published his earliest experiments on nerve tract degenerations following lesions of the cerebral cortex. In light of the poliomyelitis studies of the 1940's and 1950's, it is interesting to note that the rhesus monkey was impli-

*See also Zuckerman (1963).

cated in the earliest immunological attack on this disease by Landsteiner and Popper of Vienna in 1909.

Interest in the normal biology of the rhesus monkey did not lag far behind its first experimental use. Kinnaman published on behavior of the rhesus monkey in 1902; as did Hamilton (1911) and Yerkes (1915). During the 1920's the reproductive physiology of the rhesus monkey was widely studied by Corner (1923), Allen (1926), and Hartman (1932). The biological interest in the rhesus monkey at that time was reflected by the publication of *The Anatomy of the Rhesus Monkey* by Hartman and Straus (1933), the first comprehensive textbook on the anatomy of a nonhuman primate species.

The late 1930's saw the first attempt to study rhesus monkeys as a natural population. Dr. C. R. Carpenter exported a shipload of animals from India and freed them on Cayo Santiago off Puerto Rico. The original colony consisted of 409 monkeys; their descendants are still being studied on the island. An excellent film in color, "Rhesus Monkeys of Cayo Santiago," is available and shows many aspects of free-ranging behavior of this species.*

As Jolly (1966) states, the rhesus monkey "once entrenched in the literature . . . was ordered as a routine for future work." By 1938, 15,581 rhesus monkeys were being imported annually into the United States mainly for scientific use. The figure today is not as easy to determine as many other species are being used in laboratories, but of the 110,681 monkeys cleared for importation into the United States in 1966, it is reasonable to assume that a large proportion was *M. mulatta*.

The story of the rhesus monkey since 1940 has been associated with two dramatic discoveries: first, that of the Rh factor by Landsteiner and Wiener (1940) and second, the development of poliomyelitis vaccine in the 1950's. Equally dramatic to those concerned with primate husbandry is the steady upsweep in recent years of births and successful rearings in captivity as described elsewhere in this volume. In view of the widening experience of many laboratories in breeding rhesus monkeys it is probable that within 5 or 10 years few macaques that have not been bred in laboratories will be used for experimentation. Advances in birth engineering must move fast, faster at any rate than economic forces that are leading to the depletion of natural primate resources, if stocks are to be maintained for essential human-oriented research.

*Apply to Mr. R. Finney, Bldg. 31., National Institutes of Health, Bethesda, Maryland.

CLASSIFICATION AND TAXONOMY

The genus *Macaca* (Lacépède, 1799) comprises a group of closely related animals that are widely distributed in Asia from eastern Afghanistan and Tibet to China. Southward they extend into the Indian continent and Ceylon in the west, and to the Japanese islands and Formosa in the east. They also occur throughout southeast Asia, being found on the islands of Sumatra, Java, Borneo, Philippines, and Celebes. The species listed in Table I are currently recognized.

The crab-eating macaque (*M. fascicularis*) has been variously and incorrectly called *M. cynomolgus* and *M. irus*; neither name is available according to the International Code of Zoological Nomenclature, 1964 (Fooden, 1964). Fooden also states that *M. mulatta* and *M. fascicularis* are conspecific. This taxonomic opinion is based on the evidence of three doubtfully intermediate specimens collected in 1924. It may well be that the rhesus monkey and the crab-eater are in reality one species, but to the authors the evidence at present is insufficient. The brown stump-tailed macaque has been for many years incorrectly called *M. speciosa* owing to a misidentification of Cuvier's original description in 1825 (actually of a Japanese macaque); the correct name is *M. arctoides*, I. Geoffroy, 1831 (Fooden, 1967).

The genus *Macaca* is a category within the family of Old World mon-

Table I
Currently Recognized Species of the Genus *Macaca*[a]

Species	Common name	No. of subspecies
M. sylvanus (type species)	Barbary ape	0
M. sinica	Toque monkey	3
M. radiata	Bonnet monkey	2
M. silenus	Lion-tailed macaque	0
M. nemestrina	Pig-tailed macaque	4
M. fascicularis = (irus)	Crab-eating macaque	21
M. mulatta	Rhesus monkey	4
M. assamensis	Assamese macaque	2
M. cyclopis	Formosan Rock macaque	0
M. arctoides = (speciosa)	Stump-tailed macaque	4
M. fuscata	Japanese macaque	2
M. maurus	Celebes or Moor macaque	4

[a]From Napier and Napier (1967).

keys (Cercopithecidae). Macaques are members of the subfamily Cercopithecinae, a grouping which indicates that they are more closely related to the African baboons and mangabeys than to the African and Far Eastern langurs (Colobinae). Like baboons, macaques are largely ground-living forms and it is assumed they both evolved from a common stock of arboreal monkeys that took to living on the ground some 15–20 million years ago. Subsequently, they became geographically separated. Structurally the macaques have not become as specialized for ground living as baboons but they share a broadly similar social organization.

GEOGRAPHICAL RANGE

The approximate range of *M. mulatta* is shown in Fig. 1, while that of *M. fascicularis* is contiguous with it in a southeasterly direction and

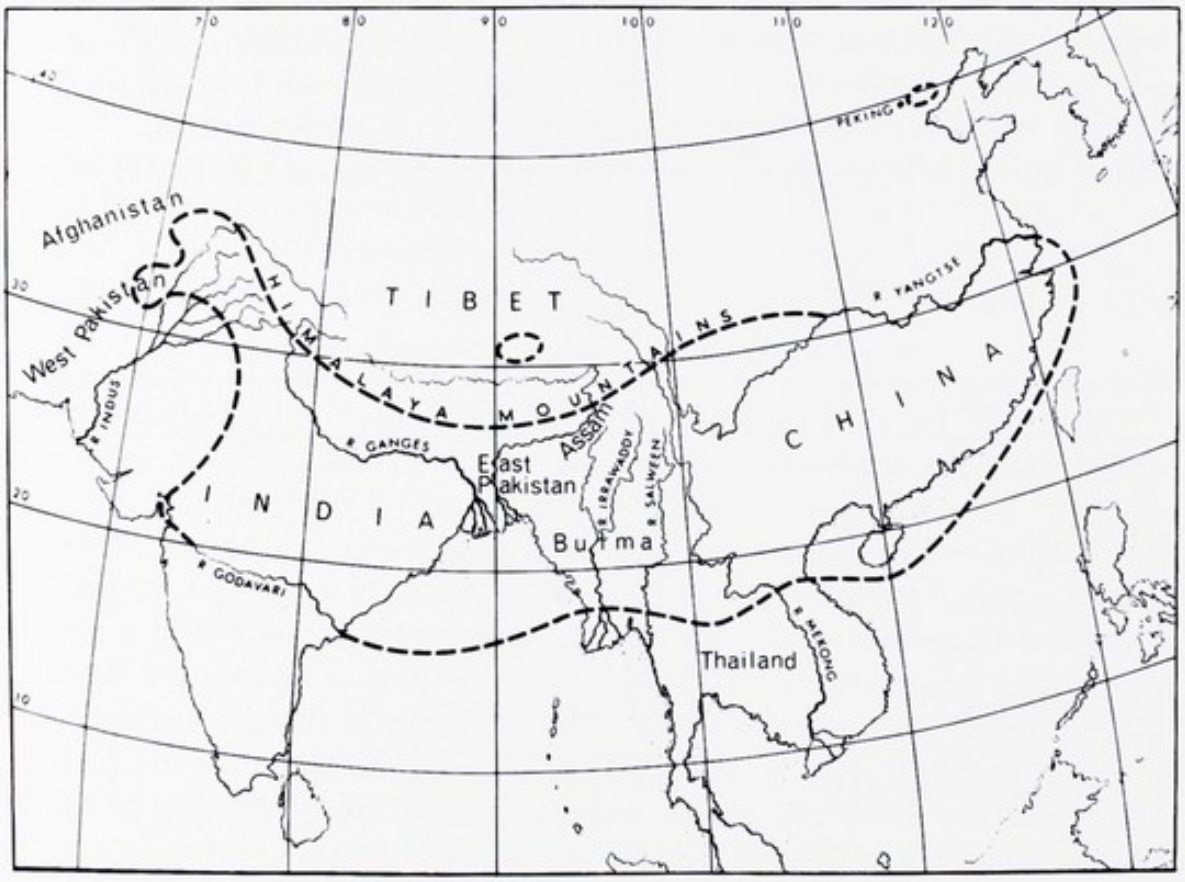

Fig. 1. Approximate geographic range of *M. mulatta*.

embraces the Indochinese and Malay peninsulas, Sumatra, Java, Borneo, and the Philippines. In terms of land area, the rhesus monkey has the greatest probable range of all macaques. It is interesting to note that in Pleistocene times the range of macaques included much of Europe and extended as far north as England.

ECOLOGY

The habitat of *M. mulatta* shows an incredible variation within its range. They occupy almost all major subtypes within the tropical biomes. They are found in tropical rain forests and monsoon forests; in montane forests of the Himalayas and the temperate forests of China; in areas of scrub and cactus, and in and around the temples and villages of India and Pakistan.

This ecological catholicity is reflected in the generalized physical structure and physiological processes of the rhesus monkey. They are omnivorous, the diet consisting of fruit, roots, young leaves, insects and grubs, native crops such as rice, maize, potatoes, and sugarcane. In terms of locomotor function, they are equally at home on the ground or in the trees; they are capable of running, climbing, leaping, or scrambling over a variety of terrains; they swim voluntarily and even the young can dive 30-40 ft into the water; given the appropriate incentive, rhesus monkeys will stand on two legs and even walk bipedally for short distances. They have prehensile hands with strong opposable thumbs and are capable of carrying out quite precise manipulations. Vital statistics for *M. mulatta* are given in Table II.

SOCIAL BEHAVIOR

Rhesus monkeys live in large groups ranging from 11 to 50 in number, in which there is usually more than one adult male. Based on a study of 399 natural groups, the average composition is as follows: adult males, 3.7; adult females, 7.7; juveniles, 1.7; and infants, 4.5. The adult sex ratio is 1 male to 2.1 females. (These figures are based on Southwick, Beg, and Siddiqi, 1965.)

Table II
Vital Statistics for *M. mulatta*

Birth season:	March–June (northern India)
Birth weight range:	330–600 gm
Body size:	
Head and body (male)	483–635 mm
Head and body (female)	470–531 mm
Tail length (male)	203–305 mm
Tail length (female)	189–284 mm
Chromosome diploid number:	42
Duration of estrus:	9.2 days
Gestation period:	164 days (146–180)
Lactation:	Lasts 7–14 months
Limb proportions:	
Brachial index	97
Crural index	92
Intermembral index	89
Sexual maturity:	
Male	4.5 years
Female	3.5 years
Weight:	
Male	5557–10,896 gm
Female	4370–10,659 gm
Zoo longevity record:[b]	21 years 6 months

[a]From Napier and Napier (1967).
[b]*Macaca mulatta* (especially those bred in laboratories) probably live much longer. On p. 100 we quote a reference to a male hypertensive of 27 years.

Clear-cut dominance hierarchies are formed between males of the group and to some extent among the females. Subordinate males usually live peripherally to the main body of the group which consists of dominant males, females, and infants, or they may be completely outside it as solitary males.

The home range of groups is approximately 3 sq miles, frequently overlapping the home range of other groups.

Communication takes the form of vocalizations, facial expressions, and body gestures; for instance the presentation of the anogenital region toward a dominant animal is interpreted as a gesture of subordination; this is referred to as "presenting." Branch or tree-shaking is commonly seen and is interpreted as an aggressive gesture. Social grooming, an important factor in group cohesion, occurs between consort pairs, females and their infants, and between two males and two females.

POPULATION DYNAMICS

The determination of present population size and an assessment of population trends are matters of urgency. The population dynamics of rhesus monkeys has been the subject of a study by Southwick, Beg, and Siddiqi (1961). These authors sampled centers of rhesus monkey population in Uttar Pradesh, a province in northern India in the middle of the species range. With regard to abundance their general conclusions were summed up in the following words: "The rhesus monkey is less abundant in villages and temples of northern India than is commonly thought." They somewhat guardedly estimate the 1959–1960 population to be less than 1 million. This ill accords with Corbett's 1953 estimate of 10 million for the same region. This need not suggest a sudden decline in the population so much as the explosion of a popular myth. Southwick *et al.*, however, found evidence of a considerable decline in natural populations which they attribute to three separate factors: (1) the diminishing tolerance of the villagers of India toward rhesus monkeys; (2) trapping and removal of juvenile males and females which deplete the potential adult breeding stocks; and (3) changing patterns of land use.

The trapping and removal of juveniles is probably the most powerful agent in the decline of populations. During 1959–1960, 14,000 individuals of this age group were being exported per month.

No other studies of population dynamics of the rhesus monkey have been carried out elsewhere as far as the authors are aware.

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II FUNCTIONAL ORGANIZATION AND STAFF REQUIREMENTS

This chapter discusses the way in which our simian colony is operated. A table of organization of our professional and nonprofessional staff appears at the end of the chapter, and includes a minimum desired staffing ratio.

THE COLONY

The colony includes, by function, four basic units – the *Adult Conditioning Colony*, the *Adult Breeding Colony*, the *Nursery* (or Infant Colony) and the *Juvenile Holding Colony*.

The *Adult Conditioning Colony* is an initial quarantine activity in which newly imported monkeys are received and kept under close observation for evidence of clinical disease. Once these diseases are controlled or irreversibly sick animals are culled, the group enters a more protracted conditioning procedure to bring their general level of health up to a standard. The adults remain in the Conditioning Colony until they can be released into the *Adult Breeding Colony* without the danger of infecting other animals.

The *Adult Breeding Colony* becomes the monkey's permanent home after it has demonstrated its competence in the breeding program. Caged individually, the monkeys are cared for daily, and observed for any signs of illness. At appropriate intervals, animals are bred. Pregnant animals are kept here until either natural or surgical

delivery. The neonate is removed almost immediately from the mother and transferred to the *Nursery*.

The *Nursery* (or Infant Colony) is the area where newborn monkeys are reared. They are placed in isolators and hand-fed by attendants. Here, the infants are cared for under carefully controlled conditions. It is at this early stage of life that the animal is inoculated with the experimental inoculum under study.

The *Juvenile Holding Colony* is a unique, controlled-environment area where young monkeys are taken after 4-6 weeks in the Nursery. Here the experimental animal is housed in a special cage providing maximal protection against adventitious infection of both the monkey and attendants. This isolated "holding area," as it is termed, would not normally be part of most simian breeding colonies and will be discussed only briefly in this guide.

SCIENTIFIC STAFF

Proper staffing of a simian colony presents problems because there is a shortage of trained professional scientists, technicians, and animal handlers. Veterinarians with experience with simians are in great demand for research programs, as are the specialized workers who must control the various supporting laboratory services such as pathology and hematology.

The shortage of professional people is most severe. Staffing problems are compounded because the colony demands around-the-clock operation. To meet emergency situations such as sudden sickness or injury to the monkeys during "off hours," an "on-call" procedure is maintained so that a veterinarian is always available.

There is a basic need for professional skills in the operation of any simian colony, no matter how large or small, but except for the veterinarians' services these may be provided by part-time participation of other professionals, depending on the size of the colony, the type of research performed, and the availability of talent.

Our colony now requires the services of four full-time veterinarians, two to care for adult animals in the conditioning and breeding colonies, the other two to care for infant and juvenile animals in the nursery and holding colonies. From the time an animal is received or born in

the laboratory, throughout the rest of its lifetime, the veterinary clinician is responsible for its health. He makes frequent examinations of all monkeys, determines their state of health, prescribes the management of ills, decides on necessary dietary variations, and evaluates diagnostic data obtained routinely on all animals in the colony.

There are three types of functions necessary to support the care of the colonies: surgery, pathology, and diagnostic laboratory services in hematology, bacteriology, parasitology, and clinical chemistry.

The magnitude and complexity of surgical support is directly related to the mission and size of the operation. The need for a surgeon depends on the sophistication of research procedures; a skilled veterinarian can handle repair of injuries, cesarean sections, and dentistry. Even monkeys kept alone in well-constructed cages may injure themselves. They are sometimes severely injured in mating, and improper techniques of handling result in broken limbs or hernias. A cesarean section is necessary to deliver a fetus in a posterior presentation or with other birth complications. We extract canine teeth from breeder males to facilitate handling. Monkeys are prone to develop dental caries and broken teeth. We generally treat severe deterioration of teeth by extraction.

Any sizable simian colony—whether used for breeding or for holding large numbers of monkeys on experiment—should have access to at least the part-time services of a pathologist who is versed in simian diseases. In all colonies monkeys will die, sometimes sporadically, sometimes by groups in epizootics. Pathological studies help the clinician to a better understanding of simian diseases so that the animals may be better treated and so that remedial action can be taken to protect the rest of the colony from a particular cause of death. Necropsies on any simian should not be undertaken in a casual manner. Precautions against infection are essential. Gloves and protective clothing must be worn and autoclaved after use. A room should be reserved for necropsy work alone and thoroughly cleaned after use. Assistants, who may be nonprofessionals, should be trained in anatomical dissection for necropsy and in the potential hazards of disease transmission.

That there is a distinct advantage in employing diagnostic laboratory services as an aid to the routine care of simians does not need to be justified here. The reliance of the veterinary clinician on these for the management of apparent and inapparent illnesses depends on the quantity and quality of the services available. We now have profes-

sional supervision of our hematology, bacteriology, parasitology, and clinical chemistry laboratories, but adequate diagnostic support can certainly be provided by competent senior laboratory technicians.

ANIMAL CARE STAFF

The recruitment and maintenance of an adequate nonprofessional staff presents a challenge because there exists no group of experienced people from which to obtain personnel.

We have attempted to solve the problem of nonprofessional staffing by an on-the-job training program and a career incentive system which encourages nonprofessional personnel to advance through prescribed levels. The program is directed by the veterinarians, but most of the training is given by senior nonprofessionals. In addition, nonprofessional personnel are encouraged to take courses such as those offered by commercial feed manufacturers, or the laboratory animal science courses offered by technical organizations.

Below the level of the supervisors, who manage the colony under the guidance of the veterinarians, the following job categories were established.

Inexperienced applicants enter the colonies as animal aide trainees. To become an animal aide, each trainee must have at least 1 year of experience and must have acquired a basic knowledge of handling, restraining, feeding, watering, record keeping, and hygiene appropriate to the care of simians, and must be familiar with the basic principles of animal care as stated by the Certification Board of the American Association for Laboratory Animal Science.

To be promoted to senior animal aide, an animal aide must have at least 2 years of experience and pass the Junior Animal Technician Examination of the American Association for Laboratory Animal Science.

An animal technician must have passed the Senior Animal Technician Examination of the American Association of Laboratory Animal Science, must be a high school graduate and have at least 3 years' experience.

A senior animal technician must have a B.S. degree or its equivalent and several years of appropriate experience at the technician level.

Except to say that the senior levels are used as much as possible for supervision and training, an exact list of duties according to job cate-

gory is not generally followed in practice. The job categories represent levels of proficiency rather than specific functions. The duties of animal handlers include: feed animals and record food intake or prepare formula for infants and feed them; clean cages, and perform housekeeping chores, including sterilization of cages; catch animals and restrain them for treatment; assist professionals in technical procedures as directed; take vaginal swabs of females and report amount of bleeding; take blood samples; collect semen from males; calculate dosages per orders of veterinarian; plot breeding cycles from menstrual records; make visual checks and written records of excreta, general activity, and appearance including coughing, sneezing, hydration, and condition of hair, skin, eyes, and external openings; give medications, orally or parentally.

Our experience has shown that hiring into the trainee classification and promoting upward is a better practice than stratifying labor as career cage cleaners, career technical assistants, and so forth. Because all animal-attending personnel must be trained into our particular procedures in all events, the policy of "promoting up through the ranks" is advantageous for morale as well as for thoroughness of training.

What makes a good animal aide? Because they are the largest category of nonprofessional personnel in our organization, we have given this question considerable thought. When recruiting new personnel to train as animal aides, we look for certain traits. The job demands someone with a regard for animals who cares enough to make an extra effort to get a young monkey to eat, or who feels enough concern to provide special attention required by postoperative convalescence. The converse of this attitude requirement is that scrupulous care must be exercised not to hire those who are malicious toward animals, individuals who not infrequently seek such employment.

A past history with animals is one of the few overt indicators that point toward success as an animal aide. We seek those who can demonstrate some actual history in nonspecific animal care, on a farm, as a pet owner, a kennel attendant, an aide to a veterinarian, or similar job.

A certain measure of physical courage is needed by those who must handle simians. Monkeys and apes are capricious animals, strong enough to do harm to humans, and frequently inclined to try. Also, they appear to sense fear in humans and react against it. Those who handle them need an extra measure of self-assurance. Although exceptional strength is not necessary, agility is important. To quickly get

control of an animal a man's strength is needed. Except for infants, which in our colony are normally handled exclusively by women, the handling of adult monkeys and apes is strictly the province of men.

Animal aides must be devoted to their work. Nothing can repair the consequences of the overdose of a drug nor heal an injury resulting from mishandling. Usually a person who is deliberate and thorough, who does not resent the paper work, and who is anxious to see it written up neatly and accurately will be an above average animal handler.

A simian colony is no place for casual employees. Animal aides should be people who want to stay on the job, rather than those who want to keep moving. They need time to build up a rapport between themselves and their charges. They learn to sense when all is well with the animals, or when it is not. They get to know something beyond what is written in the protocol or spelled out in the standard operational procedures. Therefore, it behooves the managers of a simian colony to devote extra effort to the problem of attracting and holding desirable animal handlers.

Good animal handlers are exceptional people, but they do not require exceptional education. The average high school graduate possesses enough formal education for the work, and many non-high-school graduates succeed at it. However, they must be sufficiently literate to relate certain medical terms to animal care, to know the technical names of foods and drugs used, the metric measurements employed, the names of instruments in use, and the terminology used by veterinarians to convey instructions.

All animals attendants, prior to employment, are given a physical examination by a company physician which also includes tuberculin test and radiograph of the chest. The object is to protect simians from acquiring tuberculosis from man, and also to prevent animal attendants from receiving infections from the monkeys.

Because simian infants require pediatric attention similar to that given human infants, we have found that women with children of their own are best suited for this work. Prior to caring for simian infants, a woman should have had practical experience feeding babies, burping them, changing their diapers and keeping them clean because these same things are done for simians. Beyond this, no great amount of formal education, not even high school graduation, is necessary provided they can successfully cope with a considerable number of charts and check lists.

A table of organization is shown in the accompanying diagram

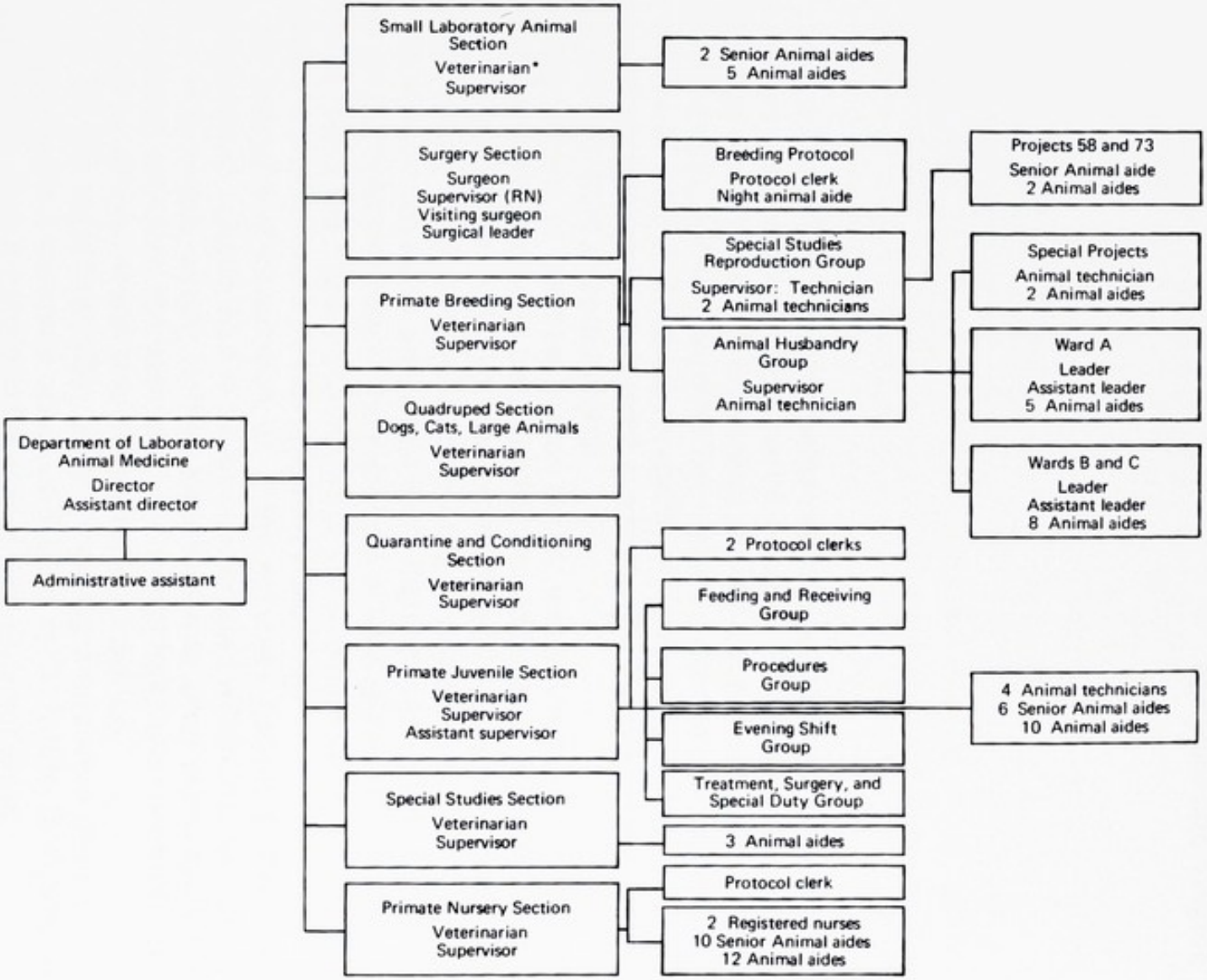

Diagram 1. Table of organization of professional and nonprofessional staff. *Each veterinarian serves more than one function.

which includes a breakdown into different sections. It should be emphasized that all work with simians is supported by a professional staff in pathology, hematology, virology, bacteriology, parasitology, surgery, and radiology.

III FACILITIES

Early attempts by many investigators to breed simians for research purposes were discouraging, partly because of the poor design of buildings and equipment. By careful attention to the design of animal spaces and equipment, we have been able to improve conception and viable birth rates and reduce morbidity and mortality. Our experience over 5 years has produced some guidelines for the design and installation of facilities and equipment.

Construction of our new simian center was started in 1964. Since then, two additions have been built to provide a total of 60,000 sq. ft. The design of the buildings has emphasized certain basic features which can be applied to the needs of other organizations.

(1) A basic objective was to achieve a high concentration of activity per unit of area because of the high cost of land in an urban location, and to make the most efficient use of construction costs. In this regard, a two-story structure has operated effectively.

(2) Because of its location, all of the activities, as well as the work flow between spaces, had to be accommodated within interior space. As a windowless, single, unified structure with good propinquity for integrated activities, the basic design concept has been successful, as judged by animal health and personnel acceptance.

(3) To the extent possible, animal holding areas were of modular design to permit alternate space utilization in the future, to minimize cost, and to allow expansion of activities into adjacent areas.

(4) Keeping within the functional requirements established early in planning, selection of materials was governed by cost and availability to permit rapid and economic construction. While there is surely no

inexpensive animal facility, economy was employed in substitution of the "satisfactory" for the "ideal" material. The concept was applied to design of facility and equipment which required substantial effort to evaluate various substitute materials.

ADULT CONDITIONING COLONY

In both the Conditioning Colony and the Adult Breeding Colony, simians are maintained in individual cages to facilitate management of illness and minimize infection. Our scientific and engineering staff designed a suitable cage for this purpose (Fig. 2). The cage is 21½ inches deep at the top, 20⅜ inches deep at the bottom, 35½ inches wide, and 35 inches high. Of welded construction, the back and one side of the cage are fashioned of 22-gauge galvanized sheet metal while other surfaces, including the floor, are heavy (8-gauge) wire. A forklift is generally used to move the 100-lb cage, although it can be managed by two men.

The cage doors are of the guillotine type and are secured at the bottom with a spring-snap latch. When a removable partition is inserted down the middle from front to back, the cage may be used to house individually two average-sized adult simians. A transparent acrylic (10 × 10 inch) window in the partition allows the inhabitants of the cage to see each other. Cages may be equipped with movable back walls, called "squeeze backs," which when moved forward ease the animal to the front of the cage for restraint and removal (Fig. 2b).

The cages are placed in stationary galvanized angle-iron racks, which are welded to iron strips running horizontally along the walls. The strips in turn are bolted through the wall to identical strips in the next room, such drastic measures being necessary to prevent the monkeys in their cages from rhythmically rocking the racks away from the wall.

Each rack is 7 ft long, 10 ft high, and contains two levels of two cages (Fig. 3). Under each level, a stainless steel drop pan catches waste material from whence it can be hosed through a common downspout into a heavy-duty waste grinder. A solenoid-operated water valve opens when the grinder is turned on. Only with a waste grinder in the line is it possible to have a 1½-inch diameter drainpipe to carry the pulverized waste into an open-site floor drain. Otherwise, monkey

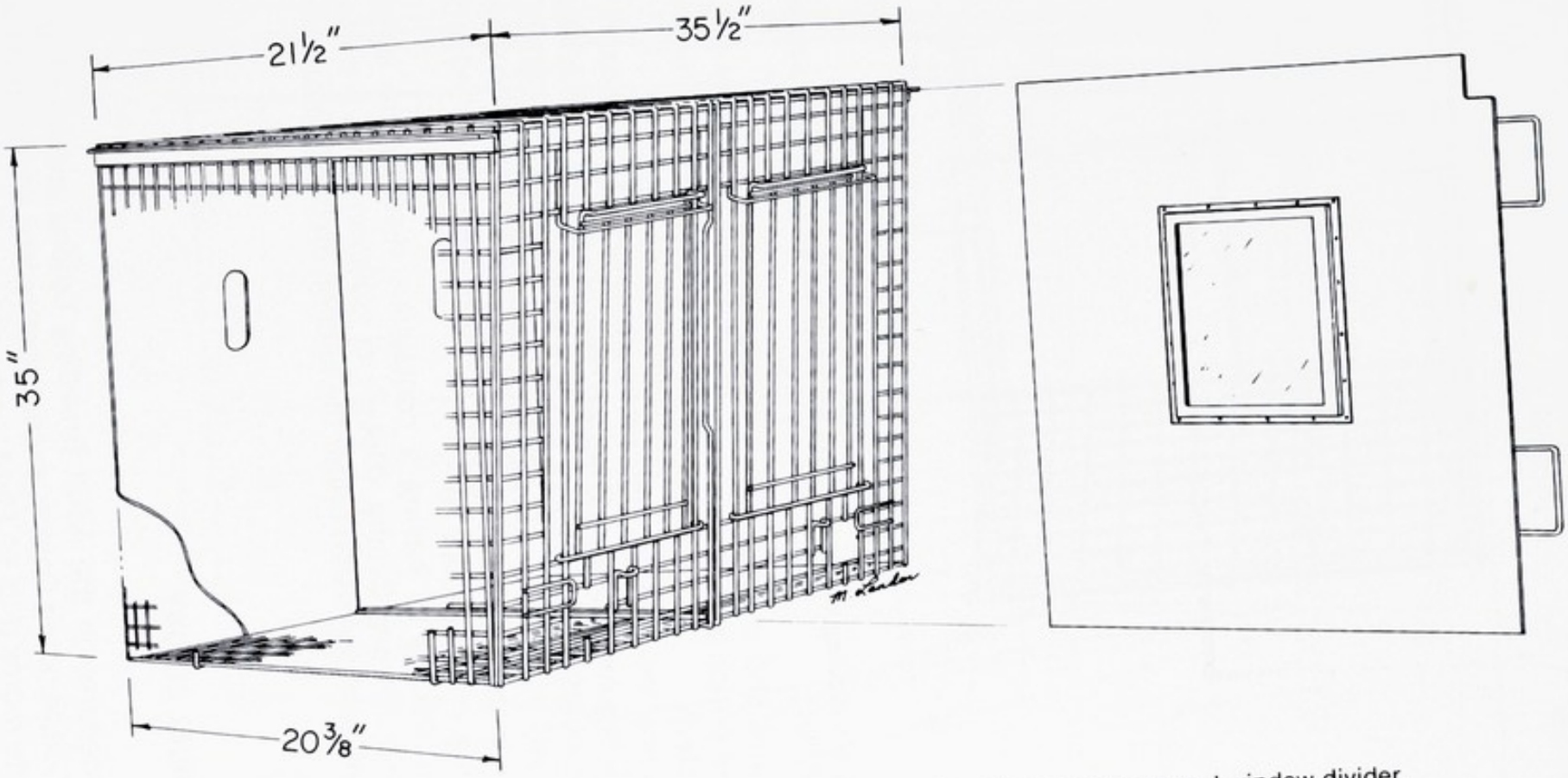

Fig. 2a. Scale drawing of holding cage for adult female monkey. Cage enclosure and window divider.

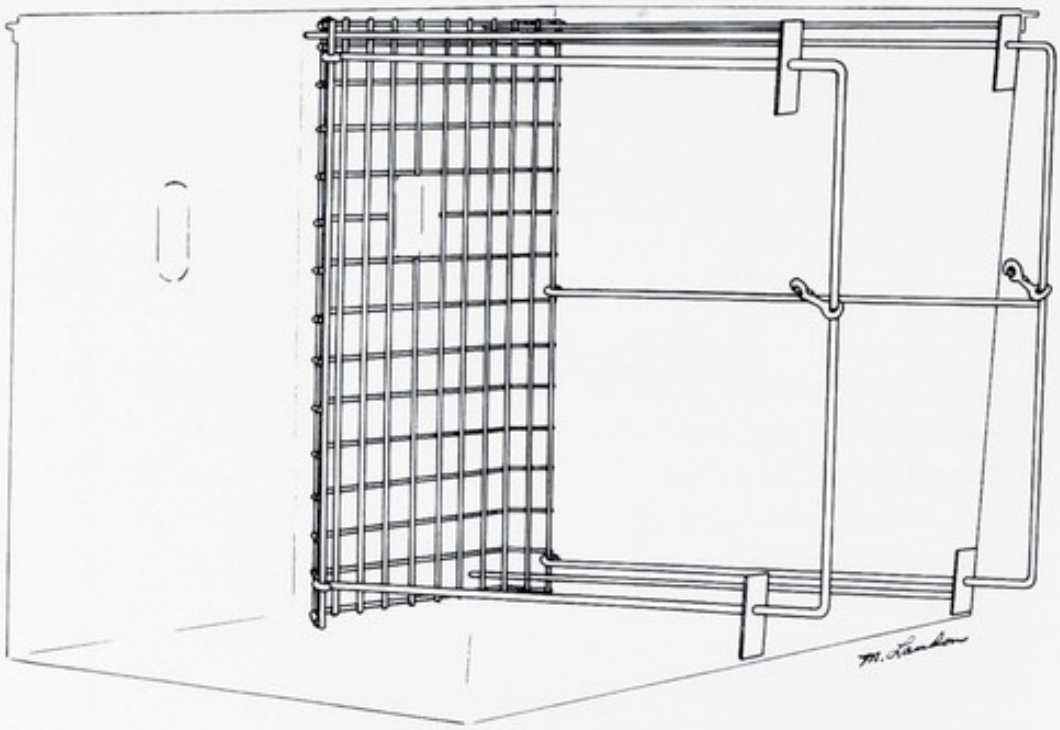

Fig. 2b. Scale drawing of holding cage for adult female monkey. "Squeeze-back" device for restraint.

chow biscuits and miscellaneous debris would invariably occlude such a small orifice and, in fact, require oversized pipes within the total drain system.

In our colony, three racks are lined up end-to-end along each side wall of a monkey room (Fig. 4). The two rows of racks are separated by at least a 3-foot aisle for sufficient room to feed, clean, and handle the animals, as well as to remove the 21-inch deep cages from the racks.

Each room in the Conditioning Colony has its own controlled airflow with room temperature about 75°F.

Animals suspected of having a contagious disease are isolated within the Conditioning Colony. (Details of isolation units are essentially the same as those listed under the section on the Juvenile Holding Colony.)

ADULT BREEDING COLONY

Caging procedures in the Adult Breeding Colony are identical to those in the Conditioning Colony, except that males and females are kept in separate rooms (Fig. 5). Eight rooms of females open off a cen-

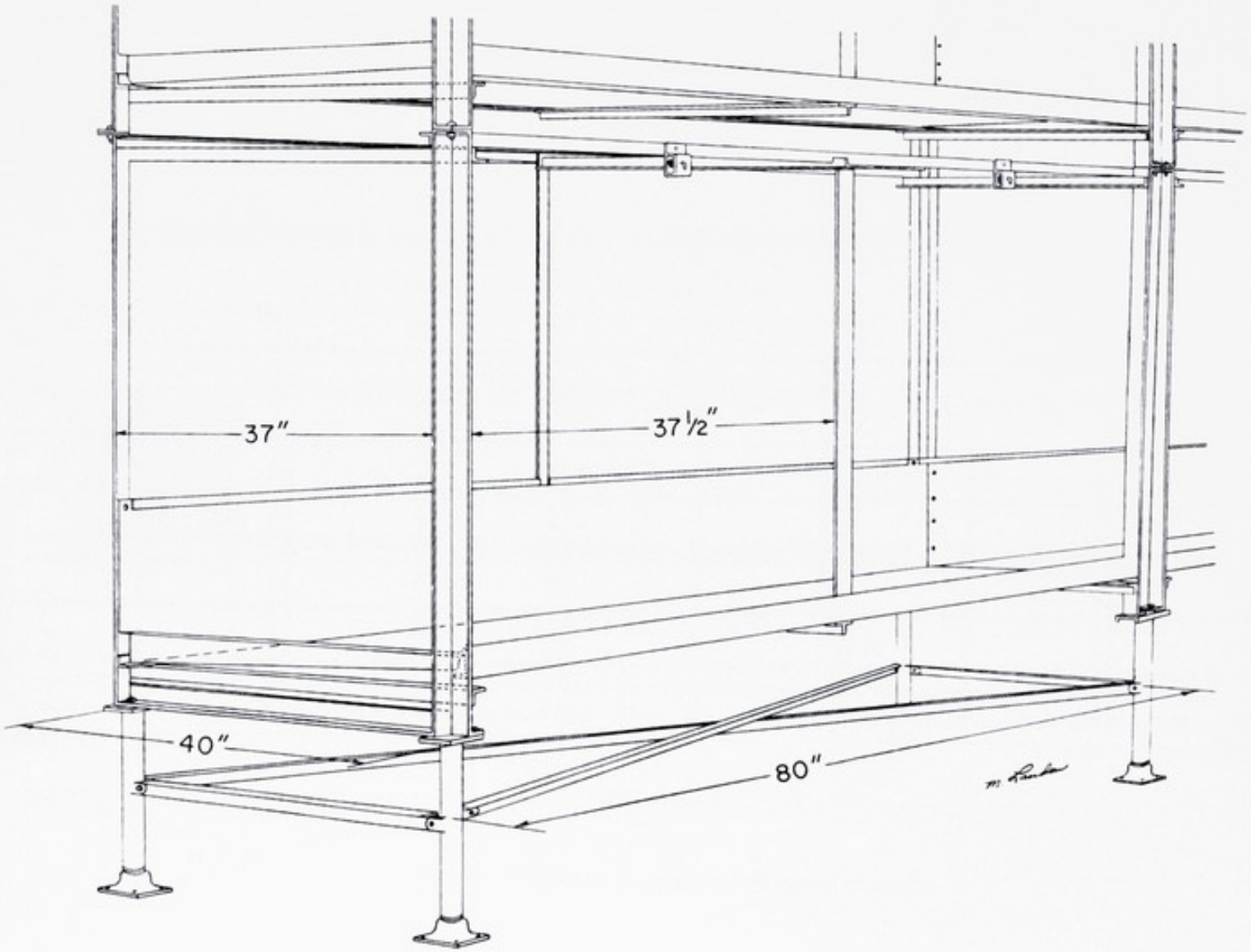

Fig. 3a. Scale drawing of two-tiered cage rack for male housing and breeding cages (three-quarter view).

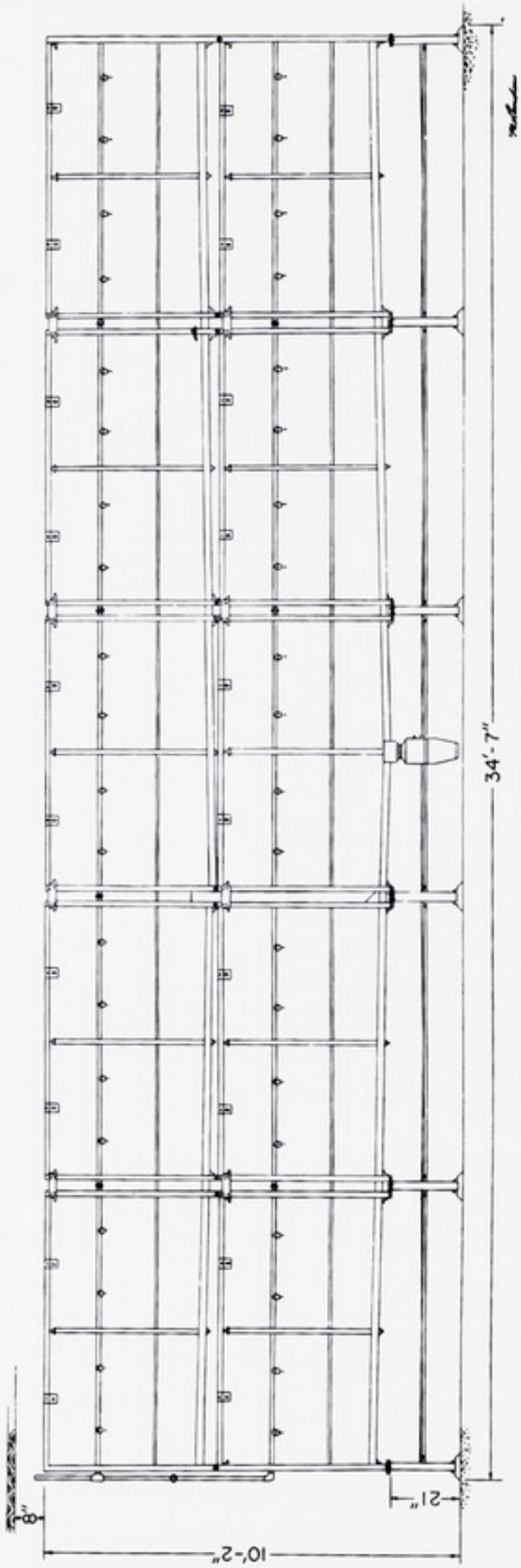

Fig. 3B. Scale drawing of two-tiered cage rack for male housing and breeding cages (front view).

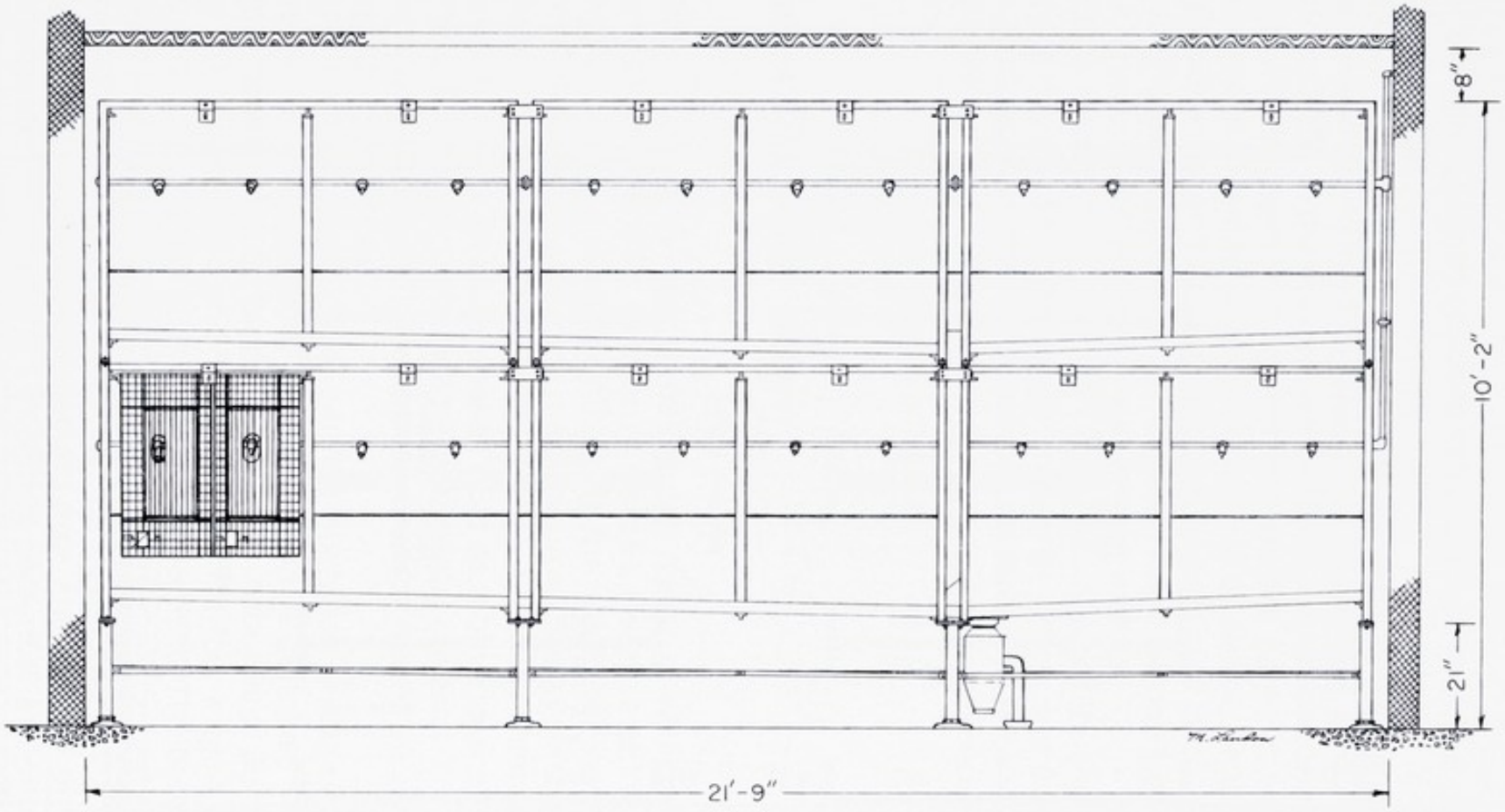

Fig. 4. Racks with cages for females in place.

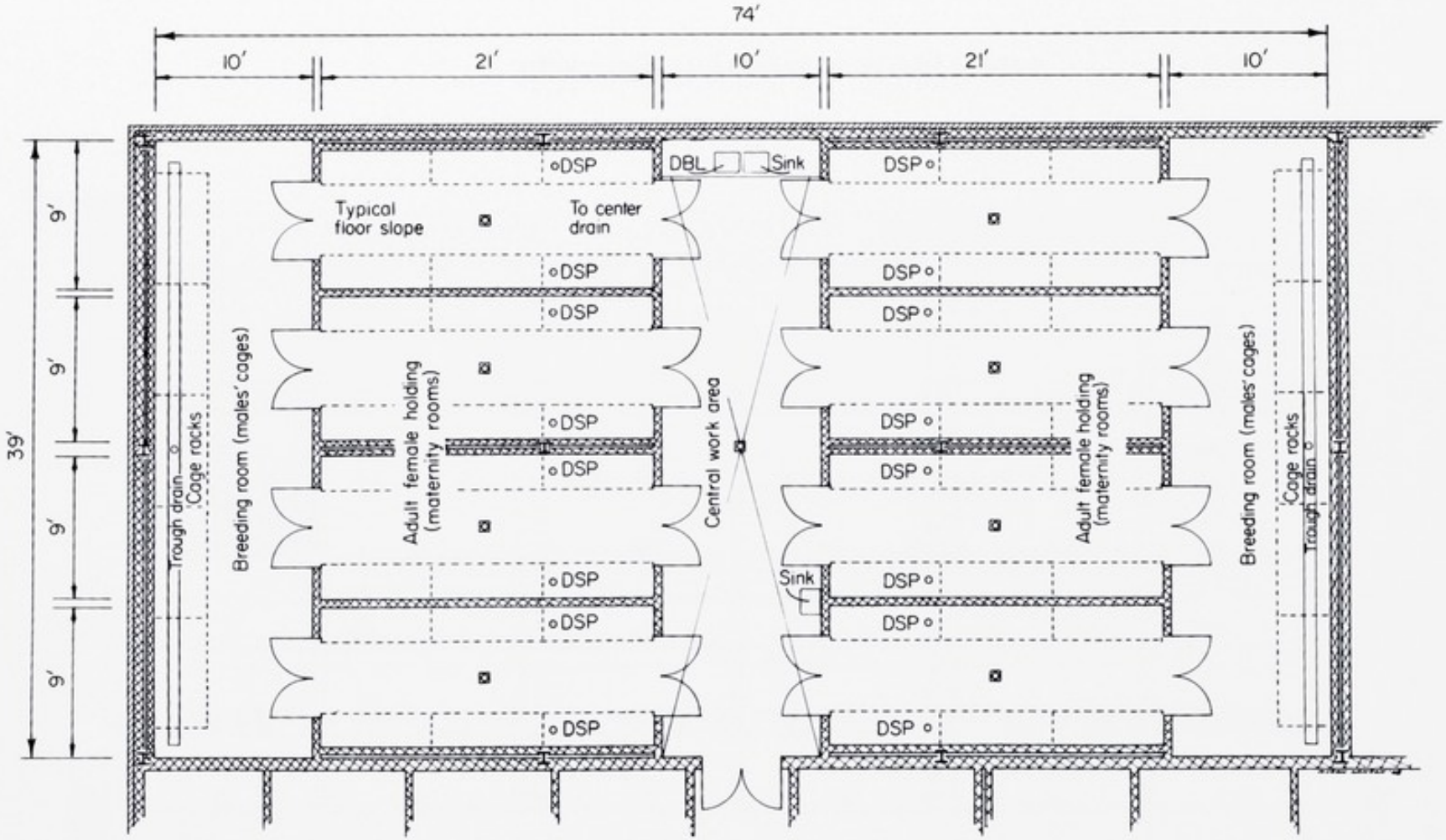

Fig. 5. Scale drawing of adult breeding ward complex. DSP, disposal.

tral work area, four on opposite sides. Each suite of four rooms opens into a common room containing males.

Females are housed two to a cage and are individually separated by a windowed partition. Each room holds 48 females, 24 on each side of a single aisle. In the male rooms, simians are housed one to a double cage with 20 cages in a room; these are the cages in which mating takes place. The male cages are similar to those used in the Conditioning Colony and in the female rooms, except that each is 14 inches deeper and is equipped with a "squeeze back" on one side of the cage to aid removal of the female monkey after mating.

Each room in the breeding colony has a separate conditioned air supply designed to provide unidirectional airflow and approximately 10 air changes per hour. The ventilation rate is necessary to minimize the chance of an epizootic disease sweeping the colony and to reduce odors.

NURSERY (OR INFANT COLONY)

Special attention must be paid to the design of equipment and the plan of floor space for newborn monkeys. Because of the potentially infectious inocula used, the infants in the colony are raised in a contained atmosphere, affording maximal protection against adventitious disease for attending personnel, as well as for the infants.

After birth, the neonate is taken from its mother, placed in an isolator (Fig. 6) and inoculated with experimental material. The isolators—specially modified germ-free types—are rigid-walled enclosures made of molded acrylic plastic.

The nonflexible structure permits operation under negative pressure (a safety precaution) and has minimal puncture liability from rambunctious infants. Each measures 50 inches long, 36 inches wide, and 30 inches high, and is mounted on a four-legged, lock-caster stand. The isolator consists of two main sections. The bottom half is a simple tublike rectangular box with a molded well at one end containing a liquid disinfectant. All objects passed in and out of the isolator must pass through this disinfectant trap. The top half of the isolator has a slanted wall to permit attendant personnel a comfortable posture for observation and any required animal manipulations inside.

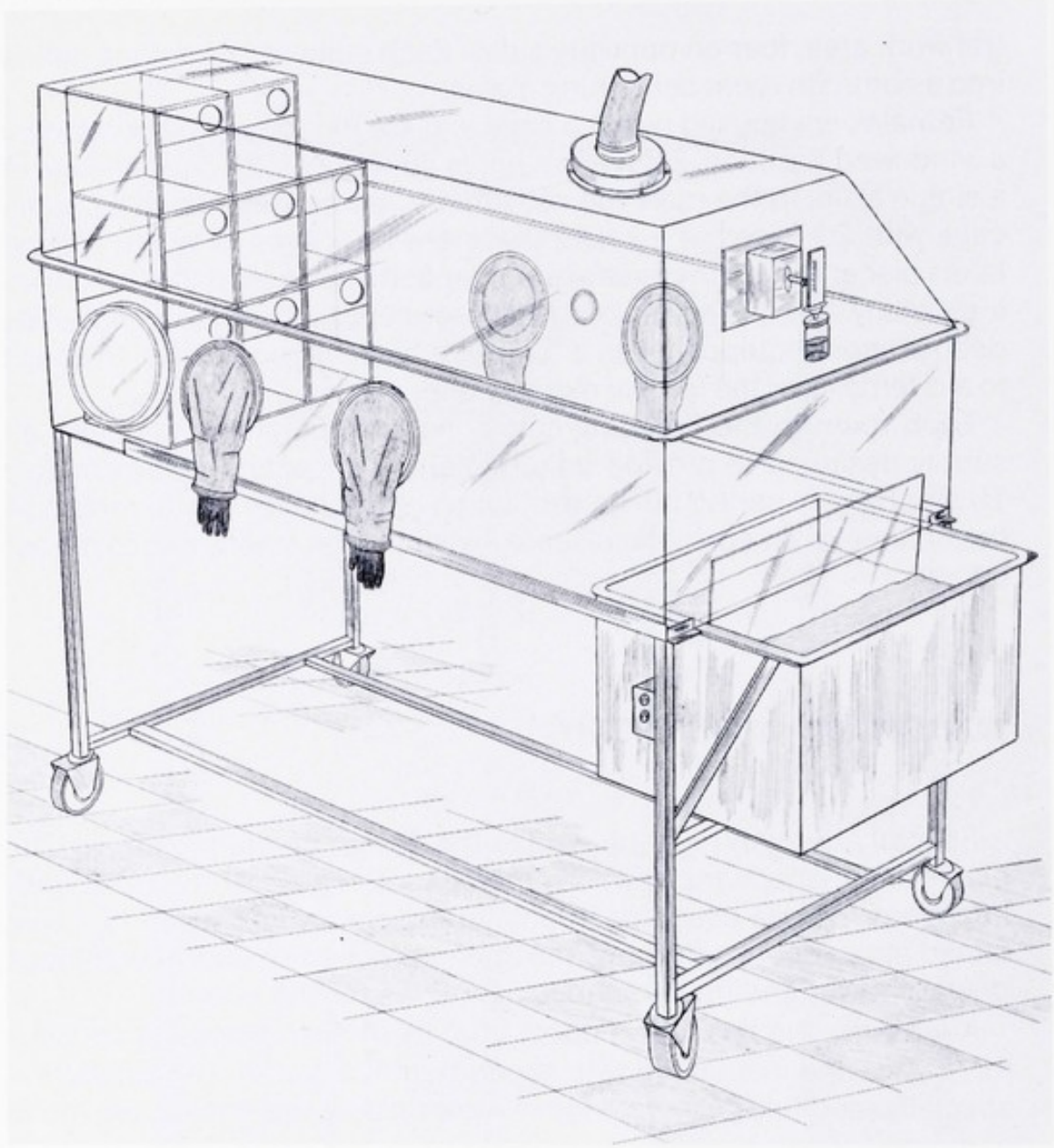

Fig. 6. Scale drawing of isolator to house infant monkeys.

The two sections are clamped together with a stainless steel cinch band and a continuous rubber gasket forms an airtight seal. Arm-length rubber gauntlets, to which necropsy-type rubber gloves are attached, are sealed into ports on each side of the isolator. These permit attendants to work with the infants from outside the isolator. A special gasketed orifice in the vertical wall between the glove ports allows the use of a stethoscope bell inside the unit while the truncated earpiece

remains outside (Fig. 7). Electrical outlets protected against moisture and peracetic acid aerosolization (for decontamination), and against the prying fingers of monkey infants, can be installed inside.

An oxygen outlet with a hospital-type humidifier is built into the isolator wall. Each isolator (Fig. 8) accommodates eight infants in separate disposable polystyrene bins that slide in and out of tiered acrylic shelves producing pigeonholes or "efficiency apartment" arrangements.

Each isolator in the infant colony is connected directly to an independent air exhaust system with the air pressure inside the isolator maintained at a slightly negative level (0.2–0.5 inches of water) with respect to the room. Air is drawn into the isolator through two layers of FG-50 high-efficiency fiber-glass media filters. Exhaust air from the isolator is filtered through the same type of filter as it is drawn into the laboratory's central "contaminated" exhaust system. The isolators are connected to exhaust manifold ducts in the ceiling of the Nursery. The isolators undergo 30 air changes per hour. Room temperature is maintained at about 75°F. Temperatures inside the isolators can be maintained at 78°–80°F by attaching standard heating pads to the undersides of the isolators.

Infants requiring special care can be raised in "isolettes" (Fig. 9) inside the isolators. These isolettes are made of two clear acrylic boxes, one inverted over another. The boxes are actually modified rodent cages, redesigned for use inside the isolator. They are slightly larger than the open disposable polystyrene bins used to cradle the healthy infants. Each isolette has a slip-on nipple oxygen connector installed in the top, and one wall is perforated for oxygen diffusion. A standard heating pad on the floor of the isolette provides needed extra heat and is regulated by nursery personnel according to a thermometer placed in the isolette.

The Infant Colony has facilities in adjacent rooms for preparing and autoclaving formula and for washing and autoclaving diapers and glassware.

JUVENILE HOLDING COLONY

At about 6 weeks of age, the infant is removed from the isolator and placed permanently in an isolated holding area. As the young simian had been inoculated with possibly infectious material, it is maintained

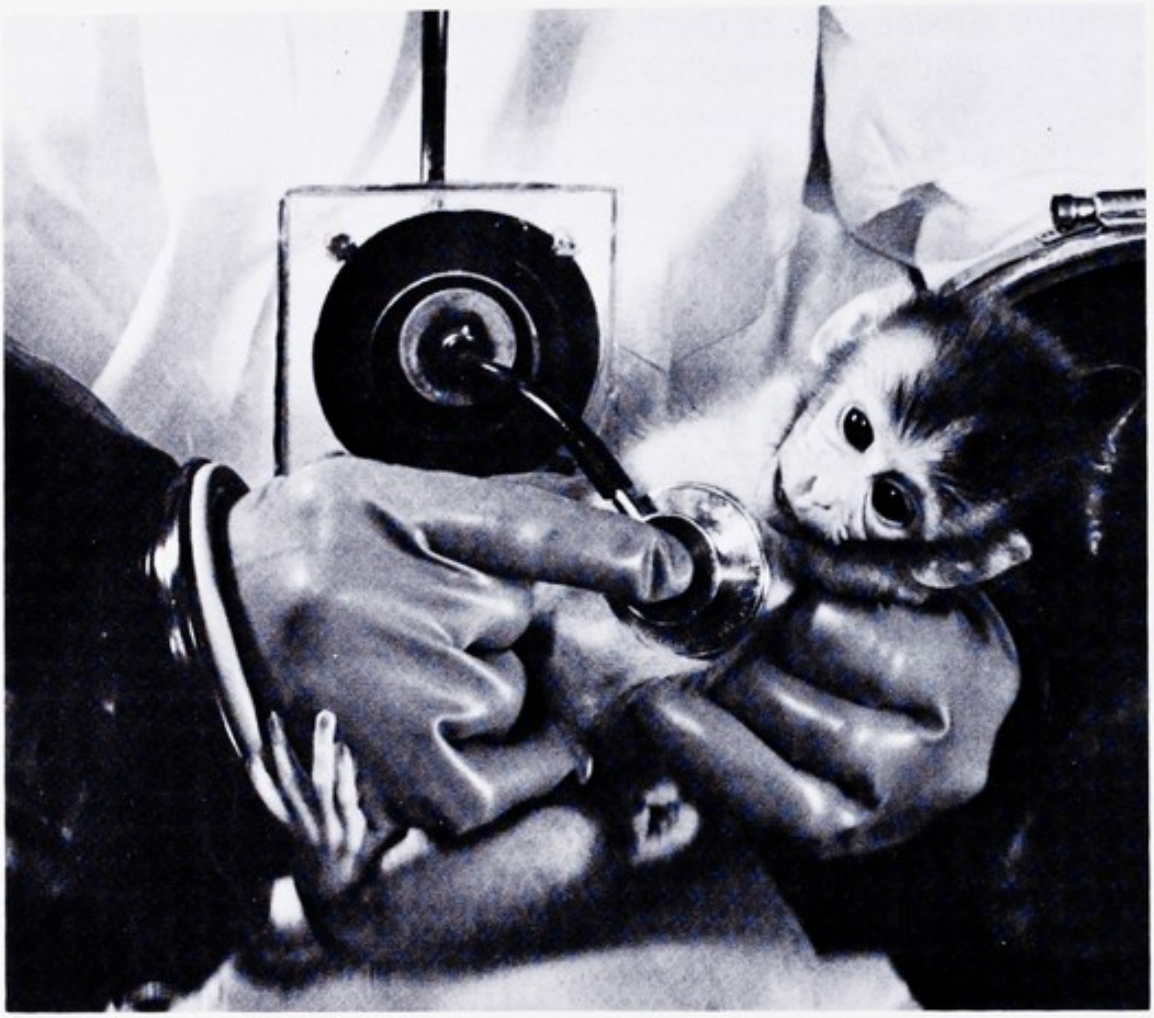

Fig. 7. Use of stethoscope in isolator.

in a contained environment; personnel assigned to its care are provided with maximal protection from exposure to possible infection.

Control of air pressure within the long-term holding area is important. The entire Juvenile Holding Colony is kept under negative air pressure with respect to all other parts of the building. The individual cages within boxlike enclosures are maintained at a negative pressure in much the same way the isolators are kept at a negative pressure with respect to the Nursery. Negative air pressure within the enclosures means that the flow of air is always from the room into the cage and therefore in a direction away from attendants who open the enclosures for cleaning and feeding.

Because of the need for negative pressure in the enclosures, each has its own exhaust port located at the back. Individual exhaust mini-

mizes the chance of cross-infection. The air supply to the holding room itself provides about 10 air changes per hour. Room temperature is maintained at about 75°F.

Personnel assigned to the isolated holding area remove street clothes in an adjacent locker room and don a long-sleeved surgical scrub suit, disposable gloves, mask, hat, and plastic boots (worn over tennis shoes) before they enter. Attendants leaving the Holding Colony disrobe in a room separate from the locker room, place their work clothes in bins for autoclaving and disposal, enter a shower for a thorough cleansing, and re-enter the locker room to change into street clothes. Air pressure increases in negativity from the corridor through the locker, shower, and disrobing rooms, and holding area to ensure air flow into the latter.

The cage and integral enclosure used in the Holding Colony are very

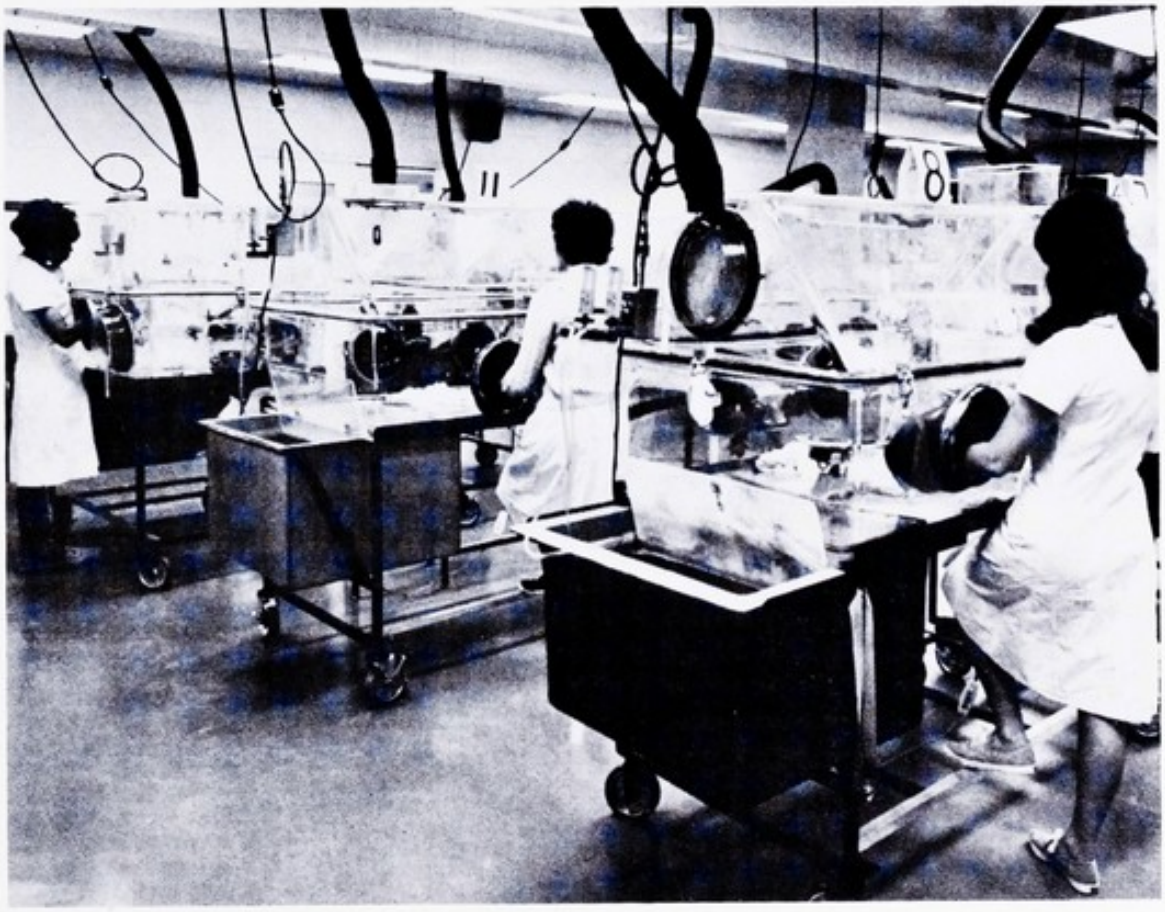

Fig. 8. View of Nursery with isolators.

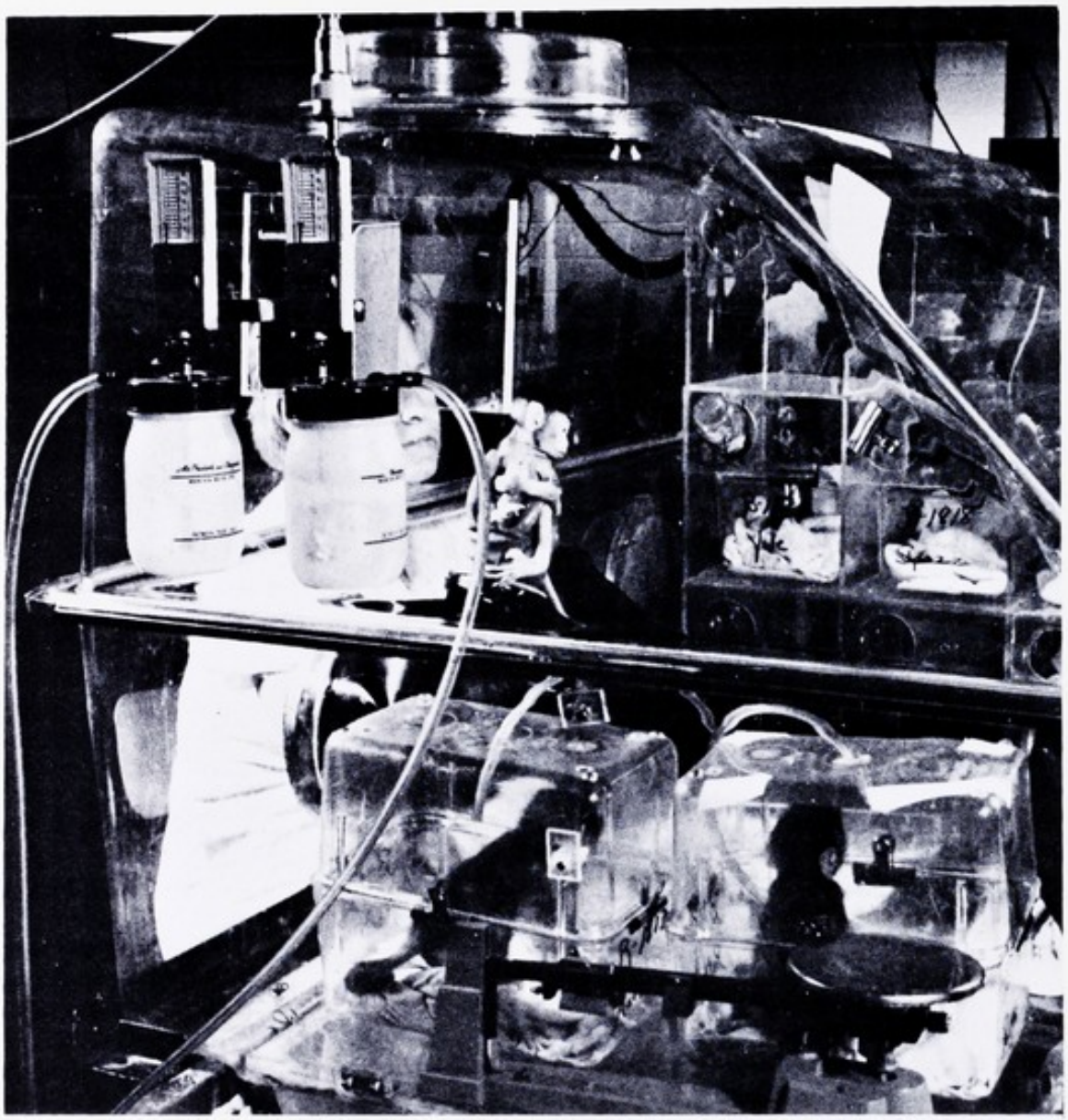

Fig. 9. Isolettes in isolator for ill or premature infants.

different from those used in the Adult Colony. Developed by us, it is called the Landon unit (Fig. 10).

The basic enclosure is made of molded fiber glass. The two door fronts are acrylic, framed with extruded aluminum angle, and each contains a filter-holder air-intake port for ventilation covered by two layers of FG-50 filter media. The cage can house one or two simians, depending on the use of a removable partition. Inside each outer acrylic door is a removable wire panel similar in design to the cage fronts in the Adult Colony. The inner wire panels have a guillotine-style

door (which can be canted outward at the top so as to clear the front edge of the enclosure when opened upward) and are kept securely in place by a spring-snap latch. "J"-type feeders are mounted on the inner wire panels of each compartment and drinking water is provided through automatic low-pressure fountains (Hardco) mounted in the rear wall of each compartment. The floor of the cage is wire. Waste is collected beneath the floor in the bottom of the enclosure and flushed out through an acid-resistant downspout and then through

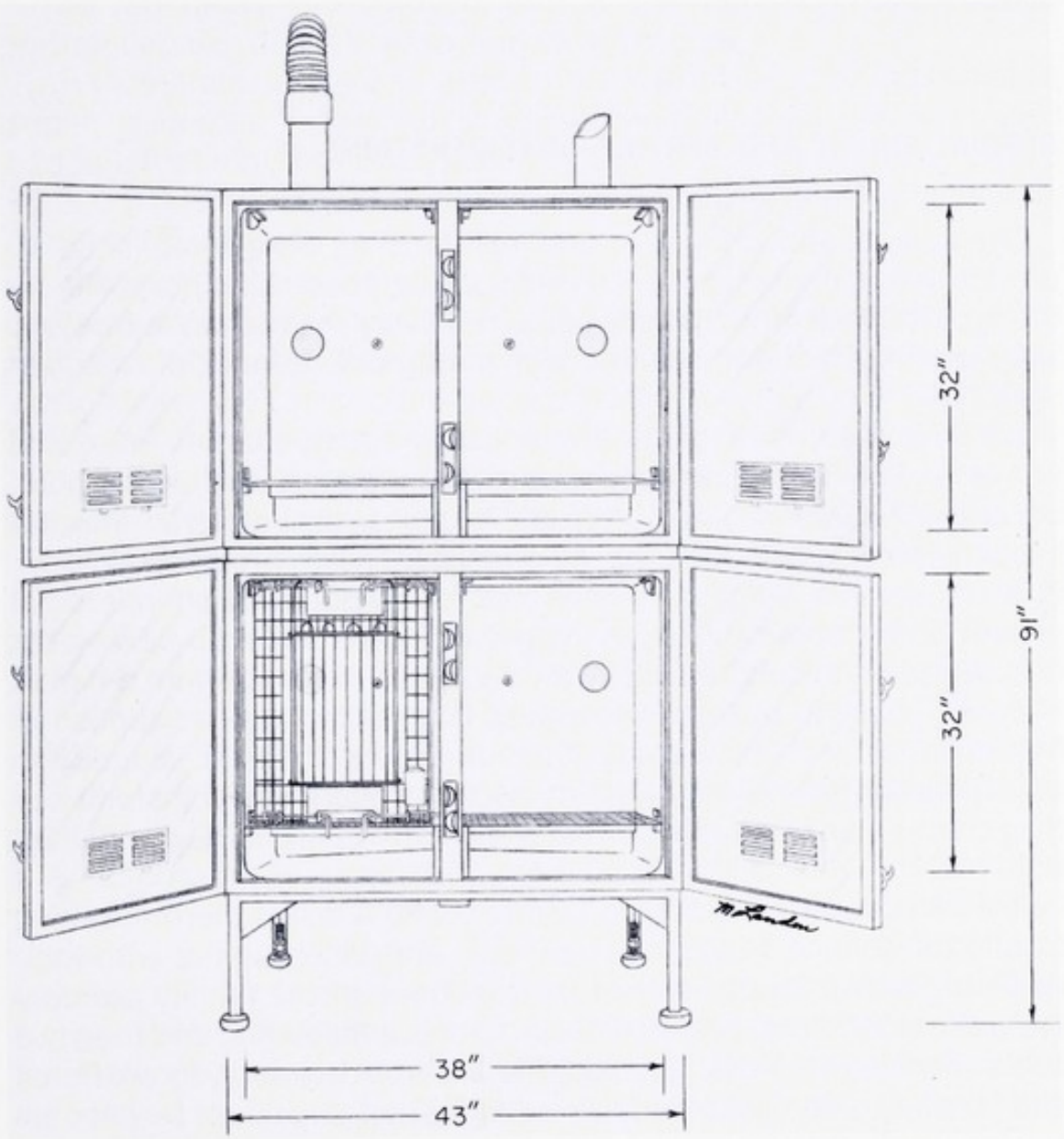

Fig. 10. Drawing of Landon unit for juvenile monkeys under controlled condition.

stainless steel troughs to the main contaminated-waste system. All waste piping is totally enclosed to prevent cross-infection of animals and to protect personnel. No waste grinders are utilized in this system.

Because all simians in the long-term holding area are potentially contaminated, ill animals must be treated within the confines of the colony. They are transferred to special fiber-glass enclosures (Kirschner). Air entering these units is prefiltered. Like the Landon unit, the Kirschner one is connected to the main exhaust system and the air pressure maintained within it is negative to that of the room. These units are equipped with outlets for administration of therapeutic oxygen.

SPECIAL FINISH AND SERVICE CONSIDERATIONS

The specifications for floor and wall finishes were established as: providing a continuous crevice-free surface; possessing durability for the type of wear anticipated; being relatively maintenance-free and withstanding frequent cleaning with strong detergent or disinfectant solutions.

We have tried many materials, including epoxy, enamel, latex, and polyester. The floor selected for use in the laboratory and animal holding areas of our facility was Dex-o-tex Neotex, a latex material, in water suspension. It is self-adhering, acid-resistant, waterproof, nonvolatile, nonimpregnable by dirt or moisture, does not support microbial growth, and is applicable so as to produce a flexible texture. Dex-o-tex is troweled on to concrete slab to a thickness of ¼ inch with a 4-inch high cove base to provide a seamless floor. It can also be applied in both exterior and below-grade areas and be patched if gouged. An advantage is its inherent flexibility because it tends to withstand settling cracks – an inevitable peril to any finish applied in new construction.

The wall covering used is Liquid Tile, a 100% solids thermal setting plastic formulated from polyester resin and incorporated with inorganic pigments. Since most of the partitions in the facility are concrete or cinder block, polyester block filler is applied first and then two coats of medium viscosity Liquid Tile are rolled or sprayed on. Pores and holes can be eliminated, provided that care is taken by the contractor. Caution should be exercised in its application to new construc-

tion; plaster or mortar must have had sufficient drying time. Premature application, when wall moisture content is still too high, can result in peeling within a year. This material resists cracking, abrasion, and puncturing, and is hard and smooth enough to withstand arduous cleaning with detergent. The material is approximately 30 mils thick of which half is block filler and the other half finish coats. On a mil-for-mil basis, it has appeared to be comparable to ceramic block.

Any sizable simian operation should be furnished with certain support equipment necessary to service its physical requirements. We have employed the following systems.

The laboratory is equipped with a standard commercial cage washer capable of wash, rinse, and steam cycles.

An incinerator (Brule) is used to dispose of dead animals, waste paper, materials suspected of being contaminated, and saturated bedding material. The unit has a 75,000 BTU primary burner plus a 75,000 BTU after-burner necessary to eliminate objectionable smoke, odor, and fly ash.

Laboratories contemplating development of a simian colony for studying potentially hazardous materials should be equipped with a high-capacity waste disposal system. We decided that contaminated waste should be carried through a welded wrought-iron drain system to the decontamination center. No vents are used in this waste system, to maintain complete isolation between contaminated areas and the atmosphere. In lieu of venting, the drain system is maintained under slightly negative pressure to the holding area to prevent backup of contaminated air and to ensure adequate flow of water. The waste material is decontaminated in two 6000-gallon blowcases. In the asbestos-insulated tanks, waste is sterilized under pressure and at a temperature, predetermined by microbiological tests, of approximately 300°F for 30 minutes. The waste is then cooled and discharged into the local sanitary sewer system. An automatic alarm warns of any breakdown in the cycle of the decontamination system.

An alternative to a central contaminated waste system is possible, provided the colony of potentially infective animals is maintained under a dry-housing system. This means that the enclosure bottoms beneath the cages are lined with an appropriate absorbent material such as cedar shavings, ground corncob, or absorbent paper. The contaminated material is removed once daily, put into garbage cans, and autoclaved for subsequent disposal. Until recently, this was the system used by us and the point at which a central waste system became

justified was simply a function of the increasingly complex logistics as the Juvenile Holding Colony size increased.

A special exhaust system for handling potentially contaminated air from holding rooms and cages is equally important. In our laboratories, all air from contaminated areas is drawn through sealed ducts to a dual bank of high-efficiency filters in the penthouse of the building. When one bank of filters reaches its maximal capacity, the flow of air is diverted to the second bank and the first bank is decontaminated, *in situ*, with steam and formaldehyde solution. After decontamination, the filters are removed and incinerated and new filters installed in their place. Both primary and secondary standby exhaust fans are provided to serve this contaminated exhaust system. The primary fan is automatically controlled to maintain a preset negative pressure in the contaminated areas of the facility. If the primary fan fails, or the load becomes too great to maintain negative pressure, the secondary exhaust fan automatically turns on and an alarm sounds.

Maintenance of constant temperature and humidity is important. A high-velocity dual-duct air system utilizing 100% outside air is used to heat and air-condition our colonies. The system provides the flexibility of individual space temperature and humidity control at any point in the building. The building is heated by four boilers. Two burn both natural gas or fuel oil—the minimal capacity to keep the facility habitable under emergency conditions. Natural gas is used as the primary fuel and a supply of fuel oil is held for emergency use. Three electrical centrifugal chillers provide chilled water for the air-conditioning system.

An emergency electrical generator permits operation of a substantial portion of the facility's services under power-failure conditions and is of vital importance in a simian center such as ours. It can be operated on natural gas or on the reserve fuel oil. Only 50% of the air-conditioning capacity is incorporated into the emergency system, the minimal acceptable capacity for the animal quarters but which does not serve the laboratory and administrative areas.

SUPPORT FACILITIES

Any simian colony must have facilities for several technical activities, and these can be successfully accommodated within the same building housing the monkeys (Figs. 11 and 12).

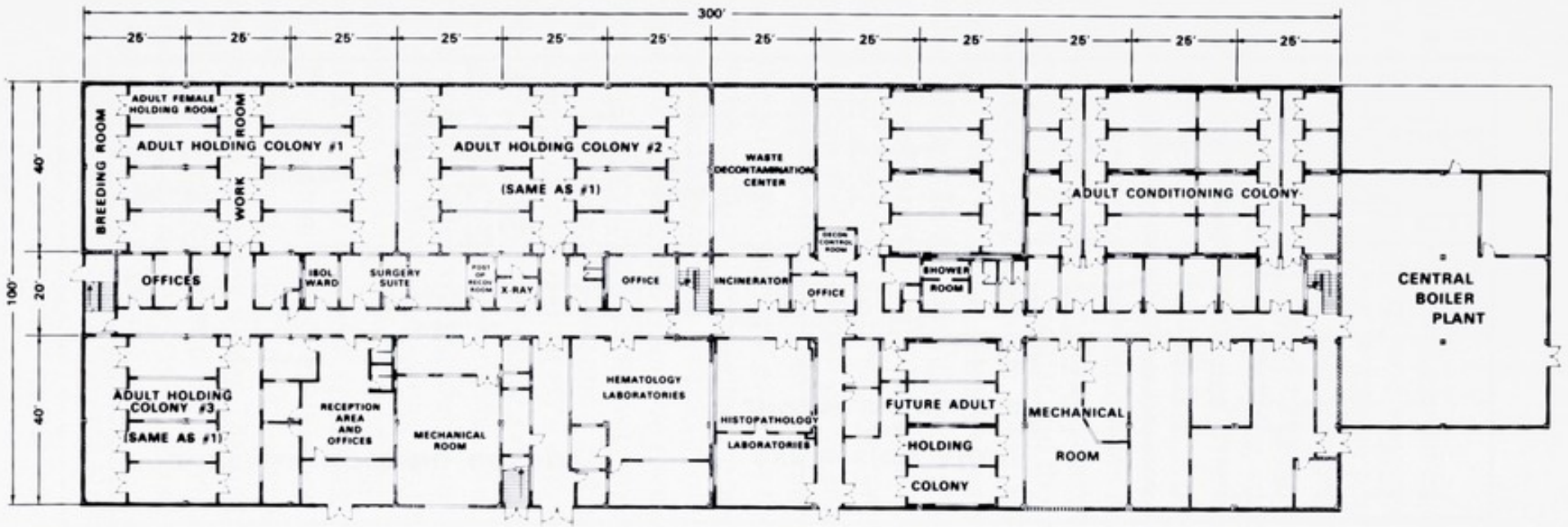

Fig. 11. Scale drawing of floor plan of simian center.

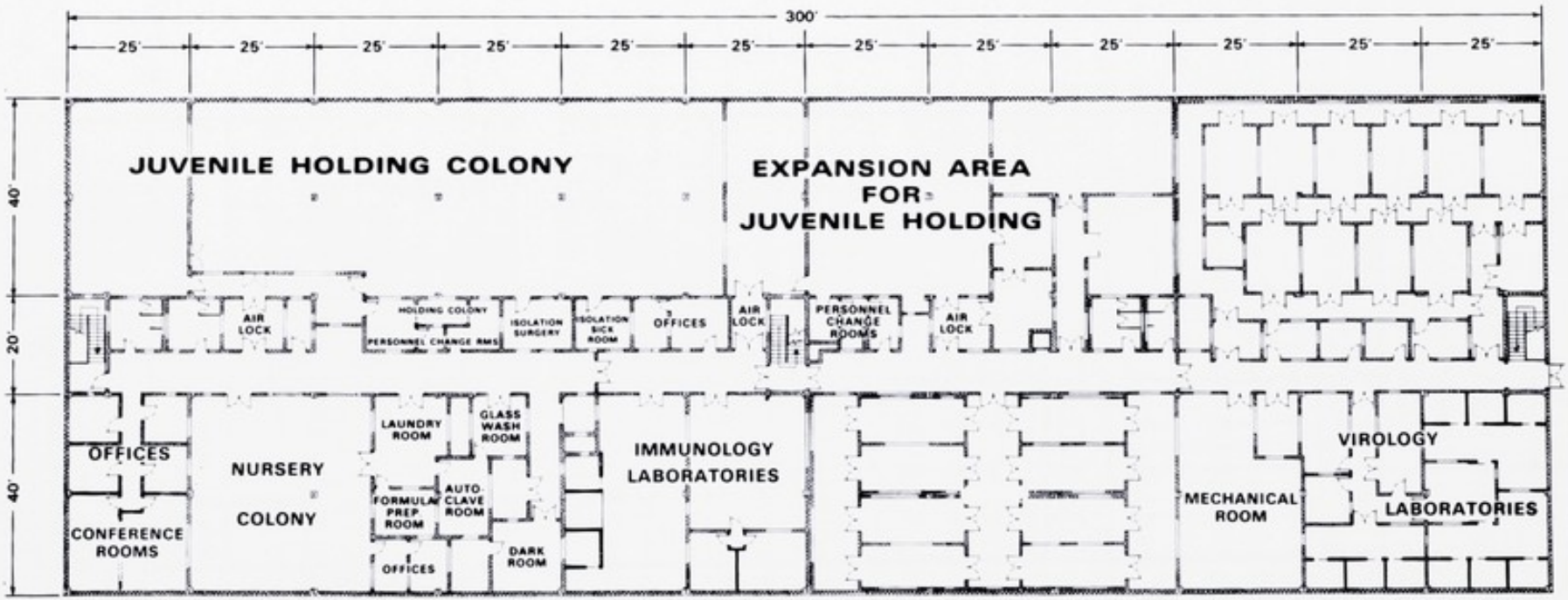

Fig. 12. Scale drawing of floor plan of simian center.

Our surgical suite is located near the animal colonies and consists of two operating rooms, a scrub room, and a recovery room. One operating room can be employed for major aseptic operative procedures, the second for minor procedures and the preoperative preparation of animals. A scrub room, located between the two operating rooms, is also used as a small record-keeping space for the surgery unit. The suite is well-lighted and ventilated. There are several outlets from a central oxygen supply in the building. A covered drain in the center of the floor of each room facilitates cleaning. Ultraviolet ceiling lights keep the area microbiologically clean when it is not in use.

An adequately designed suite facilitates maintenance of aseptic conditions throughout and permits regular and thorough cleansing for immediate disinfection of an operating room following surgery. A thorough cleansing of the entire suite is done once a week.

Another smaller surgical suite is maintained in the Juvenile Holding Colony where inoculated animals can be treated without removing them to other parts of the building. This unit has two rooms with a Plexiglas viewing bubble and arm-length gloves sealed into one of the outer walls. This arrangement permits an observer to enter the outer room adjacent to the surgical room and examine the animals through the bubble without changing clothes to enter the holding area.

The postoperative recovery room is located immediately adjacent to the main surgery unit and enables intensive care to be rendered by the personnel of the surgery unit. The recovery room contains 10 Kirschner cages, each with its own water supply and oxygen outlet. Temperature in the recovery room is kept at 80°F with a relative humidity of approximately 50%. Air is changed every 3 minutes.

A diagnostic x-ray unit occupies two rooms. One room is lined with lead sheeting to prevent scatter and accidental radiological exposure of personnel and animals in adjacent areas, and the second serves primarily as a darkroom for x-ray film processing. The equipment includes a 200-ma, 100-kv x-ray unit, with speed capabilities as fast as $^1/_{60}$ of a second, with instrument console, an exposure table equipped with an automatic Bucky grid, and thus, is suitable for table-top and standard Bucky grid high-intensity x-rays. The instrument console is shielded by a 7-ft lead-lined screen to protect the operator. The darkroom contains the standard x-ray processing accoutrements.

An isolation ward, part of the Adult Animal Colony, is provided for observation and treatment of animals showing signs of diseases, and

contains 16 modified Kirschner cages, 8 cages on opposite walls arranged 4 on 4. The room is equipped with four overhead ultraviolet lights to aid in disinfection.

The laboratories that support the clinical services are those related to pathology, hematology, parasitology, bacteriology, virology, radiology, and clinical chemistry. All are equipped with currently available automated instrumentation.

A two-room glassware preparation unit serves the various laboratories. Glassware is washed in one room containing laundry-type tubs for soaking, washing, and rinsing, drying racks, counter-top work space, and storage cabinets for clean glassware. The second room is used mainly for storage, but contains an autoclave, two stills, and a double -door drying oven.

Formula diet for infant animals is prepared in a room contiguous to the Nursery. The room is equipped with a deep-well kitchen-type sink, counter-top working space, an automatic Brewer pipetting machine for filling bottles with a predetermined amount of formula, a table-top steam sterilizer, a blender, and storage spaces for bottles, nipples, powdered formula, and other supplies. Two autoclaves pasteurized the formula.

The laundry room is immediately adjacent to the Nursery and the formula preparation room. The laundry room contains a deep-well sink (3 x 5 ft), two standard household automatic washing machines, two commercial automatic washing machines, one electric household dryer, and one gas-fired commercial dryer.

IV SIMIAN HUSBANDRY

ADULT HUSBANDRY

CONDITIONING ANIMALS

Newly imported *M. mulatta* are usually in poor states of health because of the conditions under which they are captured, held, and shipped (Ulmer, 1960; DeValois, 1960). Therefore, we set certain standards for the acceptance of monkeys into our colony, and quarantine new monkeys in a Conditioning Colony for a period of no less than 3 months and up to 6 months.

The following specifications are incorporated into our procurement orders:

(1) The monkey must conform to species, sex, and general weight specifications. (2) It must show no overt signs of systemic disease or significant physical abnormality and remain alive for 7 days after arrival. (3) It must have negative tuberculin tests before being shipped and after arrival. (4) Pregnant monkeys must be guaranteed to be pregnant and not to abort within 3 days following arrival.

Upon arrival, the shipment of animals is evaluated against the procurement specifications. The monkeys are given physical examinations by a staff veterinarian. The physical examination consists mainly of an interpretation of findings revealed by weighing, inspection, palpation, and auscultation. The general condition is evaluated by observation for the following: weight loss, dehydration, emaciation, deformi-

ties, lacerations, diarrhea, nasal discharge, labored breathing, ectoparasites, and skin lesions.

The mouth is examined for herpetic lesions of the lips, tongue, or gums. Monkeys found with open lesions are killed to preclude the spread of B-virus infection to other animals and to handlers. Dental eruption and tooth wear are evaluated as good criteria for approximation of the age of the animal. The mucous membranes are inspected for palor or icterus indicative of anemia or liver damage. The chest is auscultated for abnormal lung sounds indicative of pneumonia. Genital organs of both sexes are palpated to eliminate monkeys grossly unsuitable for breeding purposes. Males are examined for undescended or atrophic testicles and testicular masses. Females are examined for cystic ovaries, ovarian masses, and an enlarged or misshapen uterus, which may be indicative of metritis, fibrosis, retained placenta, or endometriosis.

Substandard animals are rejected and returned to the importer. The accepted animals are then retested for tuberculosis, given antibiotics for treatment of infectious disease, and injected with a vitamin-B complex. Monkeys are dusted with Diryl to combat external parasites —usually lice (*Eutrichophilus setosus*) and occasionally mites (*Sarcoptes scabiei* and *S. mutans*) and ticks (*Haemaphysalis* sp. and *Rhipicephalus sanguineus*). An identification number is tattooed on each monkey's chest (Fig. 13).

Intestinal helminths and protozoa are generally encountered in adult monkeys and a survey by fecal examination of 550 recently imported adult *M. mulatta* showed that 318 (58%) carried intestinal helminths and 259 (47%) carried intestinal protozoa*; many had multiple infections. These findings must be qualified because some of the animals may have been treated previously with anthelmintics by the animal supplier. Probably, the incidence of parasitic infestation in untreated animals is even higher than our survey indicates. Fecal examinations are carried out by the conventional flotation method, using a sodium dichromate solution of specific gravity 1.270. The helminths found, in decreasing frequency of occurrence, were: *Strongyloides* sp., *Oesophagostomum* sp., trichostrongylids, *Trichuris trichiura,* spiruroids, *Ternidens deminutus,* and *Bertiella studeri.* Intestinal protozoa are examined directly in wet-mount preparations with saline and Lugol's iodine. In decreasing frequency of occurrence, protozoa found were: *Entamoeba coli, Iodamoeba buetschlii, Trichomonas hominis, Chilomastix mesnili, Entamoeba histolytica,* and *En-*

*See Reardon and Rininger (1968).

Fig. 13. Adult female *M. mulatta* with tattoo number on chest.

dolimax nana. Thibenzole (thiabendazole), 100 mg/kg, is given by gastric intubation as an anthelmintic. This is repeated 2 weeks later. At least two post-treatment fecal examinations are made – weekly starting 2 weeks after the last treatment – to determine the efficacy of therapy.

Newly imported pregnant females are examined by intrarectal palpation of the uterus to confirm pregnancy and to estimate the age and viability of the fetus. Techniques for palpation of the uterus and a correlation of uterine size with stage of gestation are given on p. 69. The animals are given Delalutin (hydroxyprogestrone caproate), 125 mg, intramuscularly every 72 hours for a maximum of three injections and observed twice daily for signs of vaginal bleeding, which usually indicates impending abortion. Females in the Conditioning Colony experiencing a normal pregnancy are examined monthly. If the female gives birth while still in the Conditioning Colony, the infant is taken immediately from the mother and placed in an isolator in the Nursery. If the infant is removed immediately and subjected to the bathing procedure described on pp. 45-46 there is little danger that it will carry disease to other infants in the Nursery.

A survey of imported mature females indicates that animals that are pregnant when they arrive at the laboratory have less chance of survival than nonpregnant animals. As seen in Table III, over 35% of the imported pregnant animals died within 3 months after arrival. This is a significantly greater loss than the 23% of nonpregnant imported animals that died within the same period. In addition to those that died, others in each group were excluded during the conditioning process because of tuberculosis, culled as potentially poor breeders, or used for purposes other than breeding. The data indicate that, without considering the advantage of rapid reproductivity, importation of nonpregnant mature females is a better choice for the establishment of a long-term breeding colony (Valerio *et al.*, 1968).

Monkeys in the Conditioning Colony are kept in individual cages and given the same basic diet (commercially prepared primate chow) as monkeys in other colony divisions. In addition, they are given apples, oranges, and other fruits. The monkeys are weighed every 2 weeks and observed daily for clinical signs of disease. Complete records of observations and treatments are kept for each animal.

Monkeys are tested for tuberculosis every 2 weeks until the otherwise acceptable animals of the shipment have passed three successive negative tuberculin tests. Thereafter, the group is tested at 4-week intervals until the end of the quarantine period. Tuberculin-positive monkeys are removed immediately from the Conditioning Colony and killed for necropsy.

Before a monkey is considered conditioned for transfer to the breeding colony (or transfer into other colonies for research use), the following minimal criteria must be met:

Table III
Viability of Imported Pregnant Compared to Nonpregnant *M. mulatta* Obtained for the Breeding Colony

Disposition	Status of females on arrival		*p* Value[a]
	Pregnant	Nonpregnant	
Dead within 3 months	261	147	< 0.01
Dead after 3 months	21	12	< 0.01
Sacrificed (tuberculosis)	45	17	< 0.01
Introduced into breeding colony	296	362	< 0.01
Otherwise used	86	89	–
Total received	709	627	

[a]Chi square.

(1) Three months in the Conditioning Colony. (2) Five successive negative tuberculin tests. (3) No evidence of hematological abnormality. (4) No evidence of overt disease, e.g., enteritis, pneumonia. (5) Eliminated or significantly reduced parasitic infestation as demonstrated by two fecal examinations after treatment.

DIETARY CONSIDERATIONS

Adult monkeys are fed 200–300 gm of standard monkey chow (Ralston Purina or Wayne) twice daily, morning and afternoon. The food is placed in a stainless steel pan attached to the front of the cage. Water is available from an automatic watering device (Lixit) at the rear of each cage.

All adult animals are given a specially prepared vitamin-mineral supplement. The nutrients are mixed together in a blender to form a paste which is then spread between two slices of white bread to make a vitamin sandwich. Each animal receives one-quarter of a sandwich each day. The vitamin sandwich formulation is as follows:

To one gallon (3800 ml) of Pervinal syrup is added 250 capsules of Duo-C.V.P. vitamin preparation. Each capsule contains: citrus bioflavonoid compound, 200 mg, and ascorbic acid, 200 mg. To this is added 4800 mg folic acid and the mixture is blended in a Waring Blendor. One gallon of the above mixture provides approximately 700 doses which represent: 5.5 ml Pervinal, 0.35 capsule Duo-C.V.P., and 6.0 mg folic acid. Each vitamin sandwich quarter has been calculated to represent a daily supplement of: vitamin A, 2860 USP units; ascorbic acid, 71.5 mg; vitamin D, 572 USP units; vitamin E, 2.75 USP units;

citrus bioflavonoid compound, 88 mg; methionine, 33 mg; choline, 33 mg; inositol, 11 mg; thiamine HCl (B_1), 0.78 mg; riboflavin (B_2), 1.56 mg; pyridoxine HCl (B_6), 1.56 mg; vitamin B_{12} activity, 1.1 μg; niacin, 6.05 mg; *d*-pantothenic acid, 5.5 mg; calcium (Ca, 3.4%), 249.7 mg; phosphorus (P, 3.1%), 231 mg; potassium (K, 1.54%), 114.4 mg; sodium (from 3.15% sodium chloride—Na, 1.24%), 92.4 mg; magnesium (Mg, 0.22%), 10.6 mg; iron (Fe, 0.053%), 3.96 mg; copper (Cu, 0.0081%), 0.605 mg; zinc (Zn, 0.0081%), 0.605 mg; manganese (Mn, 0.0053%), 0.396 mg; cobalt (Co, 0.00030%), 0.022 mg; iodine (I, 0.00015%), 0.011 mg; folic acid, 7.15 mg.

CAGING AND CLEANING

The animals are housed individually in cage units described on pp. 18–23. The drop pan beneath each row of cages is hosed down twice daily with water and bactericidal detergent (Phenocide). Once a week, the drop pans are scraped and brushed with acid solution (Hi-Septic) to remove nitrogenous waste products.

At least once a month the cages are removed and sanitized in an automatic cage washer; however, cages occupied by sick monkeys are cleaned more frequently.

Floors of the monkey rooms and adjacent work areas are cleaned twice a day—before noon and in late afternoon. The floors are hosed down with water; then a Phenocide mixture is applied and allowed to remain on the floor for 10 minutes. The solution is removed with large rubber floor squeegees.

HANDLING THE MONKEY

The *M. mulatta*, an agile, unpredictable, and often vicious creature with sharp teeth, fingernails, and toenails, deserves the respect of all persons who work with it. It is critically important for new attendants to understand the hazards involved and to be thoroughly trained by a supervisor skilled in all phases of simian husbandry before personally handling monkeys. Scratches, puncture wounds, and lacerations—indeed, any break of the skin—can lead to all manner of infections. The possible transmission of B-virus infection to personnel can never be ignored.

Personnel must wear heavy protective clothing—particularly gloves—to protect against bites. Depending upon the task, workers should

wear heavy leather five-finger gloves that reach to the shoulder (welder's gloves with extended gauntlets), light leather gloves, rubber surgical gloves, or disposable polyvinyl gloves.

Capturing a monkey in a cage requires skill and strength. Wearing the long-sleeved leather gloves, the attendant pulls the "squeeze back" forward to grasp the animal and opens the cage. Once the animal is captured, light leather gloves may be worn to restrain it in a one-handed hold which pins the monkey's elbows together behind its back. In some instances, especially with larger animals, a handler must use two hands to effect such restraint, while another attendant performs the procedures required.

Surgical gloves are used during physical examination procedures such as rectal palpation of the uterus, and for removing infants from their mothers.

Despite rigorous observance of all precautions, bites and scratches are frequent. An attendant, so injured, reports the incident to the supervisor immediately and is sent to the laboratory's first-aid station where treatment is given by a nurse on duty, or the attendant is dispatched to a physician for more extensive care. The standard first-aid treatment for skin break injuries is a thorough cleansing with pHisoHex, a wash with hydrogen peroxide solution, and an application of tincture of merthiolate. An attendant with a bite wound receives prophylactic tetanus antitoxin if not previously immunized; otherwise, he receives tetanus toxoid. Records are kept of all injuries, including the identity of the animal and its disposition.

PEDIATRIC HUSBANDRY

NATURALLY DELIVERED NEWBORN

Within an hour after birth, the newborn simian is taken from its mother, wrapped in a diaper, and transferred to the Nursery where the stump of the umbilical cord is tied. If bleeding ensues, it can usually be controlled by gentle pressure with gauze. The infant is then given a bath to minimize transmission of infection from the mother. To bathe the infant, the attendant wets the baby with lukewarm water and spreads on 3 to 4 teaspoonfuls of pHisoHex. With additional water, the soap is worked into a lather and the creases and folds of the skin are

washed. After a rinse, the lathering is repeated before a final rinse. The infant is dried with a portable household hair dryer and placed inside a pediatric incubator (Bunn's Baby Haven) maintained at about 80–85°F. Here it is examined by the veterinarian.

Occasionally, clinical problems are encountered in the naturally delivered infant such as minor lacerations, injured tail, bruises, and umbilical hernia, most of which heal with minimal care.

CESAREAN-DELIVERED NEWBORN

Immediately after delivery and before the umbilical cord is cut, the surgeon "strips" the trachea. With the baby held upside down, the surgeon draws his fingers along the course of the trachea. This is done in a caudal to cranial direction to avoid any inspiratory gasp prior to the removal of fluids from the baby's mouth. The cord is clamped and severed and the infant placed in a bassinet resuscitator (Dann) where any additional fluid is suctioned from the trachea. Following removal of all fluid, intermittent oxygen under positive pressure is administered through a mask fitted over the face. The mask is equipped with a manual control with which the attendant can regulate the oxygen flow.

We have adopted the Apgar score method (see Table IV) to evaluate all simian babies delivered by cesarean section (Apgar, 1953). This method indicates how the infant responds to the extrauterine environment and enables one to predict its viability. Five criteria are considered to arrive at the total score. Each criterion is assigned a value of 0, 1, or 2 according to the signs observed. A score of 10 represents the best possible condition.

One Apgar score is obtained 1 minute after birth and a second, 5 minutes after birth. Survival prognosis is good if the second score is higher than the first. If it is lower, or if both scores are equally low, there is need for immediate clinical assistance. A second score of 8-10 is considered good, 6-8, fair, and less than 6, poor.

The cesarean-derived newborn is then transferred to the Nursery. The umbilical cord is tied and the infant is placed in an incubator lined with a diaper.

GENERAL HANDLING OF INFANTS

All infants are assigned a permanent identification number. Initially, identification is applied superficially using indelible ink and a system of marking employing 15 symbols on the four appendages for 60 dif-

Table IV
Criteria for Evaluation of Apgar Score of Cesarean-Delivered Neonates

Observation	0	1	2
Heart rate	Absent	Slow	160–170 beats/minute
Respiration rate	Absent	Slow, irregular	Good, crying
Muscle tone	Limp	Some flexion of extremities	Active motion
Reflex response to slap on sole of foot	No response	Grimace	Vigorous cry
Color	Blue, pale	Body pink, extremities blue	Completely pink

ferent possible markings. No two monkeys in any one age group have the same markings. Tattooing of the permanent numeric identification is delayed until the monkey is transferred to the Juvenile Colony.

At this early interval each baby receives a routine examination of the blood. Oral and anal swabs are also cultured for bacteriological and virological surveys.

All newborn infants are weighed. As indicated in Table V, the average birth weight of a baby from a laboratory-bred monkey is significantly higher than that of one from an imported pregnant monkey. In both cases the males weigh more than the females.

The baby may remain in the incubator for a matter of hours or days, depending upon its health. When it is considered healthy, it is transferred to an isolator (Fig. 14). The transfer involves placing the infant in a plastic bag with an oxygen atmosphere. The bag is sealed with a rubber band and passed through the isolator's Nolvasan (chlorhexidine) liquid disinfectant trap. During the stay in the isolator, records of body weight, food consumption, hematological findings, and clinical procedures are kept for each infant.

Many dietary regimens have been evaluated in the establishment of a feeding program for the newborn simian. We have found that their nutritional requirements are adequately met by one of the commercial human infant formulas.

Formula is prepared according to the manufacturer's directions and dispensed through a Brewer pipetting machine. The filling of a day's supply of bottles requires an hour and bottles are not refrigerated during this interval before being placed in the autoclave at 230°F

Table V
Average Birth Weight of Live *M. mulatta* by Natural Delivery[a]

	Breeding of mother	
	Received pregnant	Bred in laboratory
Male	440 ± 69	504 ± 23
Female	402 ± 87	474 ± 74

[a]Weight given in grams, ± standard deviation.

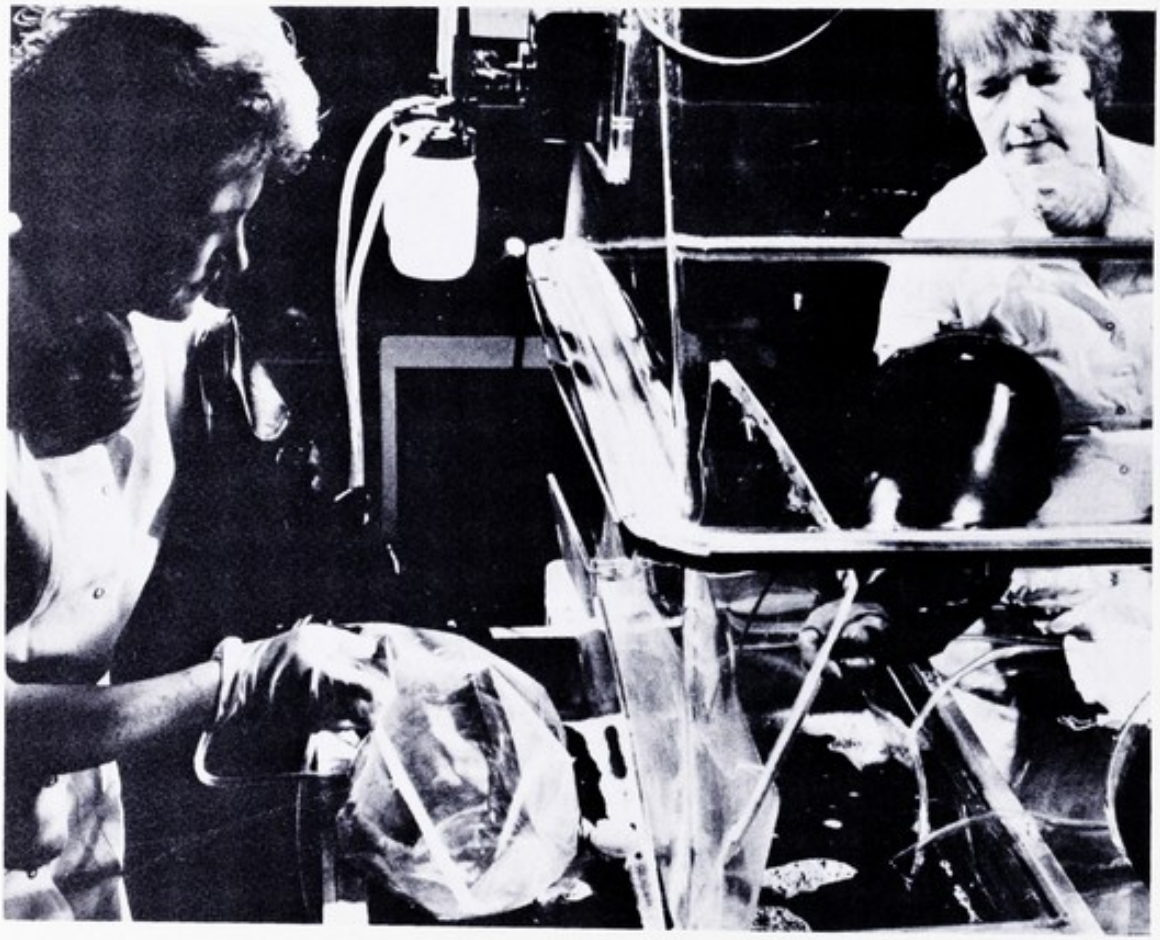

Fig. 14. Introduction of neonate into isolator.

for 3 minutes. When the autoclave cycle is completed the milk is removed, cooled, capped tightly, and refrigerated. When the day's formula is placed in the refrigerator, extra bottles from the previous day are removed, emptied, and washed with the next group of used bottles. By adhering to this procedure, it is not necessary to label the bot-

tles as to the date of preparation (although the pans should be labeled) when they are refrigerated.

The trend in the current marketing of commercial human infant formula is toward prepackaged disposable bottles and nipples. The small-sized nipple required to feed baby monkeys has limited our ability to adapt these prepackaged units to the Nursery and Holding Colony programs. The quantity of our needs is not sufficient to make special commercial preparation of these units economically feasible.

The initial feeding, within 4 hours of arrival in the Nursery, is 3-5 ml of 5% dextrose solution. If this is tolerated, approximately 2 hours later the infant is given an 8:5 mixture of Alacta and Dextri-Maltose diluted by half with 5% dextrose solution. Subsequent feedings are the Alacta-Dextri-Maltose mixture. One hundred and fifty calories per kilogram per day is the minimal guide for baby monkeys during the early period although variations may be made. Similac with iron becomes the diet as soon as the infant can self-feed and it is prepared according to the manufacturer's directions. At 2-3 weeks of age, the diet is supplemented with strained baby cereal mixed with applesauce and bananas. Several drops of Vi-Daylin in each morning's feeding are added to the fluid intake and this vitamin supplement is continued until the infant is approximately 3 months of age.

Newborn monkeys lose up to 30 gm of body weight during the first few days. Subsequently, although there is a great deal of individual variation, they gain an average 3-5 gm a day for the first few weeks; after this males gain at a slightly faster rate than females.

In the first hours and days of life the monkey is hand-fed by a Nursery attendant. The attendant wears a face mask and disposable gloves when handling or feeding the infant before it is placed in the isolator.

The baby is lifted by its body—never by the loose skin or the extremities—wrapped securely in a clean dry diaper, which quiets it, and offered a bottle (Fig. 15). For feeding, the baby is held in a vertical position, as in its natural nursing state, and is given frequent rests to allow the stomach to expand. A newborn or difficult feeder is more apt to eat if tilted slightly backward and the nipple placed in its mouth in an enticing manner. Milk flow is observed and adjusted, as in human feeding practices.

Burping is essential during the hand-feeding procedure; however, it is necessary only after a whole bottle has been consumed (Fig. 16).

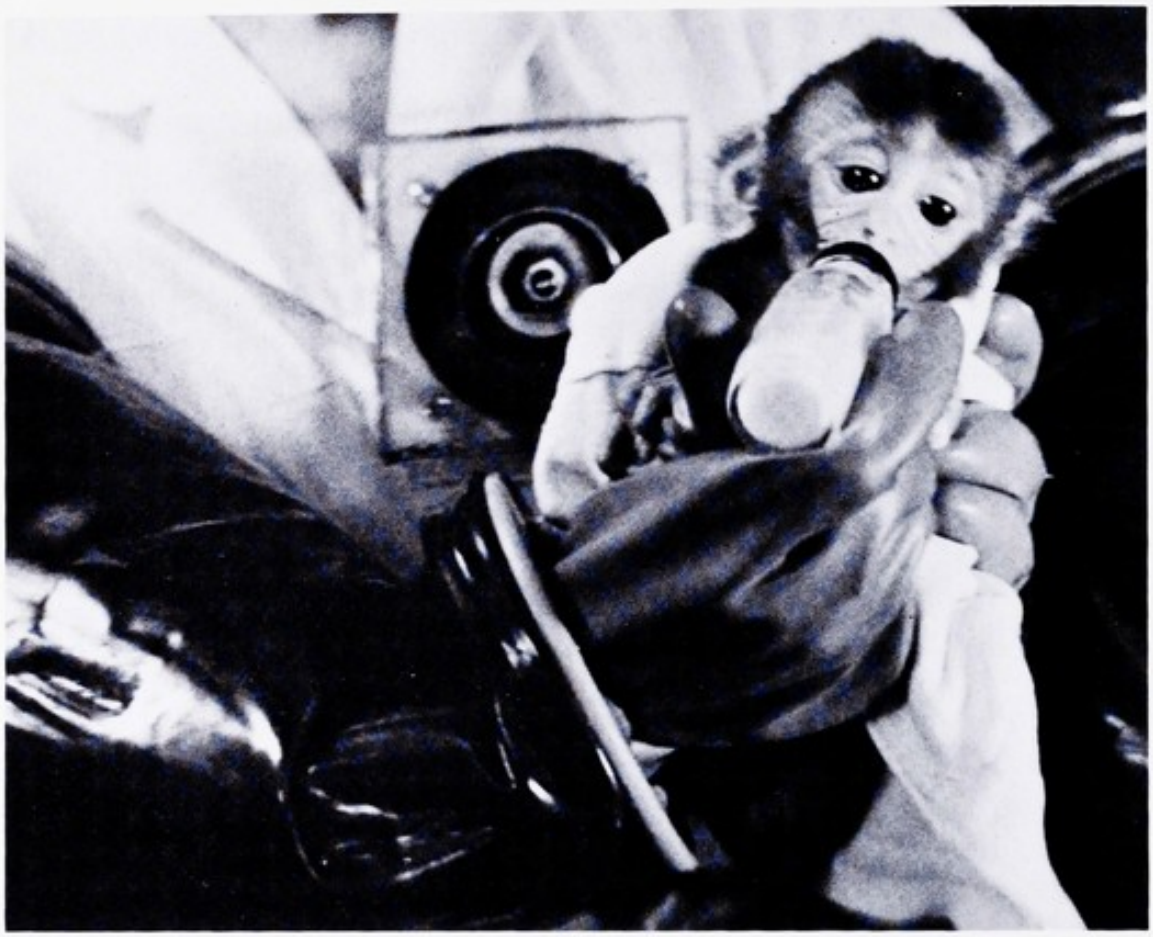

Fig. 15. Infant in isolator being bottle fed.

After the feeding, the baby is put gently back into its bin, containing dry diapers, and its head is elevated.

Infants are encouraged to self-feed as soon as possible. Some do so as early as 3-5 days. The bottle is placed in a wire-frame device attached to the bin. The infant is allowed to nurse leisurely and it generally consumes all the milk available within the first hour or two. Whereas regurgitation is common with hand-fed infants, it is negligible once they have converted to self-feeding.

Feeding by stomach tube is necessary for many premature, sick, or weak babies. A French plastic tube, approximately 15 inches long, is used for gavage of the infants. One person can complete this procedure by holding the animal, wrapped in diapers, gently restrained with the left hand. The end of the tube to be inserted is grasped in the free hand approximately 1½ inches from its end, and the tube is passed gently through the nose. When the tube cannot be passed comfortably through the nose, it may be passed through the mouth, and through

the pharynx directly into the esophagus. Some individuals have better success in passing the tubes through one orifice rather than the other, and either route can be safely employed. Difficulty in inserting the tube is seldom experienced. The location of the tube can be determined by observing the distance it has passed into the animal and watching for air bubbles from the free end of the tube submerged in water and for the cough reflex. Once the tube is inserted to the proper position, a syringe is attached to the adaptor end of the stomach tube and the technician slowly administers the food material. Following this, the tube is pinched off and removed slowly. In our experience, infants can tolerate stomach tubing procedures as frequently as every 2 hours without any ill effect.

The infant that requires gavaging is generally somewhat depressed by illness, and most animals in this condition tolerate tubing well. The animal that becomes stressed and cyanotic because of being handled and gavaged is more advisedly hand-fed. When gavage is not tolerated and regurgitation results from bottle nursing, feeding is discontinued and the infant may be maintained for a 24-hour period on intravenous or subcutaneous feeding only.

Fig. 16. Burping of infant.

Even in adults it should not be assumed that the only hazard associated with the intubation technique is that of tracheal intubation causing aspiration pneumonia. We have documented three cases in adult monkeys that had been intubated with a kaolin mixture in which a stainless steel rodent stomach tube had punctured the pharynx or cheek pouch and passed down the paraesophageal fascial plane into the mediastinum. These animals survived many months after we found that the material had produced large continuous cysts. The cysts contained the medicinal and had granulomatous walls. Radiographs suggested the presence of tumors (Valerio and Ulland, 1967).

FEEDING SCHEDULES

The infant receives its first hand-feeding within 4 hours after arrival in the Nursery. If the infant is unable to suck, or is uninterested, food should not be forced during the first 24 hours of life. As soon as the infant is able to nurse, it is fed 5-10 ml of formula every 2 hours; then 10-20 ml every 3 or 4 hours between 8 A.M. and 11 P.M. The volume is gradually increased and by the end of the first week the intake is about 25-30 ml four times a day. This regimen continues until the animal self-feeds at which time it receives 50-80 ml of milk three times daily. Once self-feeding is well established the diet is supplemented once a day with 67 gm of baby food.

Infant formula for the whole Nursery is prepared daily, put into individual nursing bottles, and refrigerated. Before feeding, the bottles are warmed in hot water. The attendant, wearing disposable sterile gloves, removes the protective nipple caps and puts the bottles into plastic bags which are sealed securely with a rubber band and passed through the Nolvasan traps into the isolators.

In the Nursery, all newborns are nursed on small nipples which are punctured with a heated 27-gauge needle. As the infant's intake and nursing ability progresses, holes are made with 22-gauge needles. As the infant gains weight and becomes better coordinated, a larger bottle with a larger nipple can be used. To ease the transition, a very soft "preemie" nipple is often used before the standard large nipple. Nipples are routinely screened and those with enlarged holes are discarded.

After feeding, empty bottles are again placed in plastic bags which are passed from the isolators into the Nolvasan traps where they soak for approximately 15 minutes. The bags are then removed from the

traps, placed in "G.I. cans," and delivered to the steam room for autoclaving at 230°F for 30 minutes. Because of an irreducibly high incidence of bottle breakage and the hazards of injury, we have used plastic feeding bottles of the 4- and 1-ounce sizes. After autoclaving, nipples and caps are separated from the bottles, placed in separate bins, and the bottles washed with soap (Alconox) and water.

Diaper bedding provided in the infant bins for comfort, absorbency, warmth, and security are changed as often as required, but at least three times daily. Fresh bins are provided once daily. The soiled diapers are packaged in paper bags and placed in plastic bags for removal from the isolators. The paper bag prevents the melted plastic from adhering to the cotton diapers when they are autoclaved. They are autoclaved at 250°F for 1 hour, washed, dried, repackaged, and autoclaved prior to return to the Nursery for isolator use.

Indicators are used to ensure the maintenance of effective sterilization procedures. A Diack sterility indicator is used when autoclaving at temperatures of 250°F, or higher as in the case of clean and dirty diapers. One Diack is placed in every fifth bag of clean diapers.

All infants are examined and weighed daily (Fig. 17) until they leave the Nursery for the Juvenile Holding Colony.

A weekly hematological examination consisting of a complete blood count is made; if the hematocrit falls below 35%, a reticulocyte count and platelet count are made. Clinical chemistry and bacteriological examinations are made as indicated and appropriate therapy is instituted.

PREMATURE INFANTS

The characteristics that distinguish a premature infant *M. mulatta* are a weight of less than 300 gm, a sparse coat, general inactivity, and sometimes a lack of muscle tone. Often premature babies delivered by cesarean section (although there are exceptions) have a low Apgar score. The problems of prematurity are inversely proportional to the degree of fetal development.

The premature infant is placed in an incubator maintained at 85-90°F. If unable to nurse, it is fed by gavage with 6 ml of formula every 2 hours. If vomiting occurs, the formula is diluted or the diet is temporarily changed to a dilute electrolyte solution (2 parts of water to 1 part of the Ambex electrolyte formula). Alcohol, sugar and, Cosa-Terramycin may be added to the electrolyte solution as warranted.

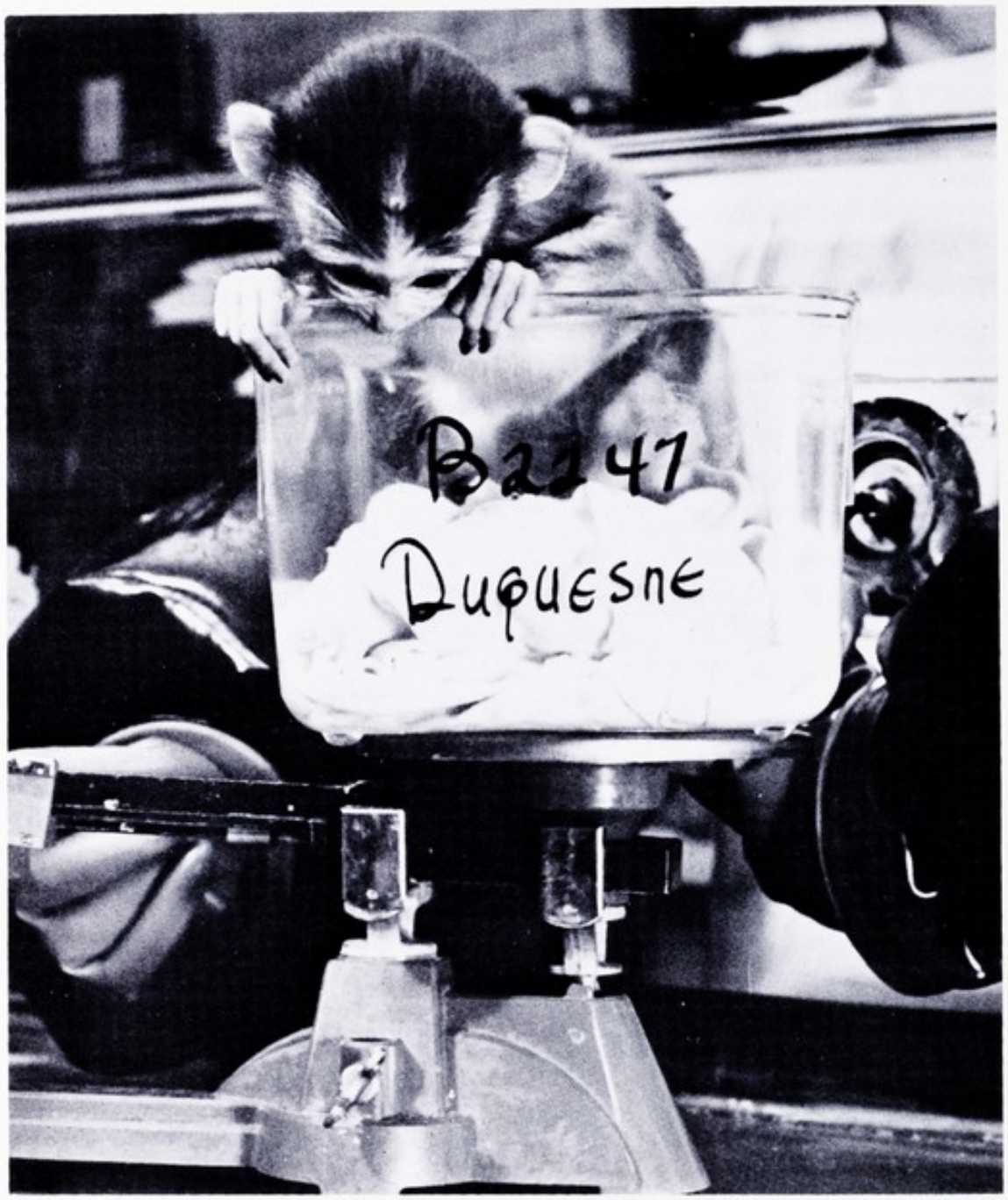

Fig. 17. Weighing of infant in isolator.

There is no routine procedure for feeding and treating premature monkeys. Each one must be considered individually. Babies that survive 14 days, accepting and responding to gavage feeding, usually show some weight gains. These infants can be bottle-fed by hand, 10 ml of formula every 3 hours. In 20 days, self-feeding is attempted and a favorable prognosis can be inferred if prematures reach this point.

At this stage, the essential difference between these and normal term infants is the smaller size.

Oxygen and antibiotic therapy is always incorporated in our clinical handling of the premature monkey. Oxygen is given at the rate of 4 liters per minute which, in our incubator system, provides as high as an 80% oxygen atmosphere. Retrolental fibroplasia has never been detected in infants so maintained for prolonged periods. Without oxygen therapy, most prematures have difficulty in breathing which, even in the normal infant, is mostly abdominal. The prognosis is guarded in the premature (or full-term) infant that remains cyanotic even under increased oxygen tension. The cyanosis may be indicative of incomplete expansion of the lungs, which usually results in death within 24-48 hours. Chest retraction is often observed only for a few days and is not regarded as serious except in an obviously moribund patient. Antibiotic therapy includes Longicil which is given at the rate of 30,000 units/kg every 72 hours. If specific pathogens are isolated from throat cultures, antibiotic sensitivity tests are completed following which the drugs of choice are substituted. Occasionally, 10-20 ml of 5.0% dextrose, or a combination of 10 ml of 2.5% dextrose and half-strength lactated Ringer's solution, given subcutaneously, provide desirable supportive care. The use of injectable multiple B vitamins and iron contributes to a healthier premature infant.

Physical activity is usually a sign of health in the full-term infant but is often the reverse in the case of the premature infant. The thriving premature appears comfortable almost continuously and awakens only when interrupted for feeding or administration of drugs. Hyperactive premature infants appear bright, but seem to wear themselves out and are prone to respiratory distress. Overt signs of illness are usually noted in hyperactive infants after 3-4 days of life, leading to a progressively debilitated state, ultimately fatal. It is not unusual for the life of the moribund infant to be prolonged for 10-15 days although, even to the inexperienced observer, the infant is obviously dying.

TRANSFER FROM THE NURSERY

At 30-60 days of age, depending upon its health, growth, and development, the baby monkey is transferred to the Juvenile Holding Colony. At this time an identification number is tattooed on its chest.

Physical examinations are conducted weekly on every monkey, whatever the age. Hematological examinations are made monthly of

all animals up to 1 year of age; after that, they are made at quarterly intervals. Tuberculin testing is initiated when the juveniles are 3-6 months old and is carried out monthly thereafter. Complete records are kept of these tests as well as of food consumption and clinical procedures.

Once the infants enter the Holding Colony, they are placed two in a cage equipped with a heating pad (Fig. 18). They are maintained in this manner for 2-3 weeks until acclimated to the new environment. The Holding Colony is maintained at about 75°F. As the infants mature, they are housed singly.

The feeding schedule established in the Nursery is continued in the Holding Colony. As the animals mature, a greater quantity of baby

Fig. 18. Landon units in Holding Colony.

food is added to the diet along with fresh fruit. At 3 months of age, commercial monkey chow is added to the diet. The monkeys are gradually weaned from milk and baby food at 4-6 months of age.

The routines followed in the Holding Colony differ to some extent with animal age. Body weights are obtained on animals 1-6 months of age at weekly intervals; 6 months to 1 year at monthly intervals; and thereafter, on a quarterly basis.

INFANT MORTALITY

In our program, a significant number of infants die before they reach 1 year of age, and over 50% of the deaths occur within the first 20 days of life. After this, there is a marked decline in the mortality rate. As shown in Table VI, the mortality rate is higher among infants born from imported pregnant monkeys than among infants born from laboratory bred monkeys.

The numbers in Table VI include *M. mulatta* exposed to various experimental inocula and those that were not. Many of the latter (sometimes euphemistically referred to as our control group) were animals either premature or weak, or otherwise not worth the investment of an experimental inoculum. The steady decline of the death rate, despite the extensive inoculum program, is testimony to the experience gained in all aspects of simian husbandry and medicine.

Table VI
Deaths within First Year of Life of *M. mulatta*
Born between May 16, 1964 and May 15, 1967

Year	Conceived in laboratory	Conceived in natural habitat	Total deaths
1964-1965	35 of 77	39 of 67	51%
1965-1966	49 of 208	14 of 46	25%
1966-1967[a]	30 of 308	13 of 29	13%

[a]The numbers for this year have excluded 90-odd infants born within our colonies which were used for an irradiation program.

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V SIMIAN REPRODUCTION

Candidates for breeding are selected at the time of receipt and are further evaluated during their stay in the Conditioning Colony. Breeding animals should be in good physical condition and of a sexually mature age as judged by fully erupted canine teeth. The males should weigh no less than 8 kg, the females no less than 4 kg. The males should pass semen evaluation within normal limits; the females should show no gross pathology of the uterus and ovaries as determined by intrarectal palpation. The vagina and cervix must be normal based upon examination with a vaginal speculum.

We have found that these criteria are reliable, although not infallible, in the selection of sexually mature monkeys capable of participating in a breeding program. In our colony, there is a ratio of one male to eight females.

THE BREEDING FEMALE

It is well documented that many simians exhibit menstrual phenomena similar to those of women, as contrasted with other female mammals; that is, there is no special recurrent period of estrus but instead, there is a menstrual cycle temporally similar to that of human females. Ability to accurately predict the time of ovulation is important for an efficient breeding program. This is not easily accomplished in monkeys. Measuring body temperature in monkeys is laborious because they are uncooperative. Moreover, we consider this to be an unreliable criterion for determining ovulation because a monkey's

body temperature may rise because of excitation as a result of handling. For a large colony, extensive laboratory analyses of the estrogen and progesterone levels in blood or urine are not a practical way to determine the phases of menstrual cycles.

Another method, described by Hartman (1932), involves a quantitative study of the cell content of the vagina. There is an undulating rise and fall of the white blood cells during menses followed by a preovulatory sharp drop and spike. Later, there is a gradual but rather rapid descent to zero white blood cells at which time ovulation is presumed to occur. This method is quite useful in determining the time just prior to ovulation, but does not indicate whether ovulation has or will occur. In applying this quantitative cell count method, mating should occur when the neutrophils have not yet reached, but are approaching, the zero mark. In theory, ovulation should occur at the zero point, but we have always found a short time lag. The quantitative study of vaginal cell content is a laborious and impractical procedure to do routinely in a large breeding colony.

The method we currently use for estimating the time of ovulation is determining the length of the menstrual cycle. In the normal, healthy *M. mulatta*, ovulation occurs approximately 12-13 days after the onset of menstruation. This is empirically based upon impregnations of females during this time interval.

The periodic changes in the sex skin of the *M. mulatta* are not valid indicators of either ovulation or menstruation. The sex skin is a secondary sex characteristic and reflects estrogenic activity by an edematous thickening and brilliant reddening of the skin over the external genital region, over the rump and tail, extending down the leg often to the knee; these changes are often accompanied by a bright reddening of the face. Our observations indicate that although it is most extensive in young females, as a rule it fluctuates as to presence, extent, and time of year in a very unpredictable manner. In older females the sex skin is less pronounced and the redness may persist for longer intervals. A good description of these changes is given by Corner (1923).

The most practical method we have found for determining menstrual cycle lengths is to obtain daily vaginal swabs from all females in the Breeding Colony. This swabbing procedure is necessary because, occasionally, the menstrual flow is occult and recording only the presence of blood on the trays below the cage is unreliable. The swabbing, performed by an attendant familiar to the animal, is accom-

plished by inserting a cotton-tipped swab into the vagina and then recording the macroscopic menstrual hemmorrhage on individual records according to the following grading system: 3+, marked; 2+, moderate; 1+, slight; and −, none. Most females become accustomed to this daily procedure and present their external genitalia to a familiar attendant. The vaginal swab procedure is usually instituted after the animal has spent 1 month in the conditioning program. Some animals are not immediately responsive to this technique and perseverance is essential. On occasion, they can be encouraged by following the procedure with a treat such as a raisin or a sugar cube. We record the onset and duration of menses, the degree of menstrual flow, and the length of the menstrual cycle.

Normal menstrual cycle length among *M. mulatta* in our experience, as well as that of many other investigators, ranges from 26-32 days with a mode of 28 days. We have observed extreme cycle lengths from 16 to 50 days. Menses usually last from 2 to 5 days, although extremes from 1 to 8 days have been observed (Valerio *et al.*, 1969a).

When impregnation occurs the menstrual cycle is abated. Vaginal bleeding can be detected at the approximate time of the next cycle which is the phenomenon of implantation bleeding discussed later. Following parturition or abortion, menstrual cycles can be expected to recur in 1-3 months. Females have conceived as early as 45 days after parturition and have experienced a normal pregnancy. Because in our laboratory the females do not nurse their young, we have had little opportunity to observe the effect of lactation on resumption of the menstrual cycle.

THE BREEDING MALE

Mature simian males, if not effectively managed, are hazardous. The threat is not only to caretakers but also to unreceptive female monkeys. Some time ago, the canine teeth of several particularly aggressive males were extracted to make them less dangerous. As neither their sexual behavior nor competence was affected, the procedure has been adopted as a routine for all Breeding Colony males.

Wounds inflicted by males on female mates at unpredictable times and in unpredictable fashion can be severe. A major problem is bite wounds which are not easily observed when the female is in the breed-

ing cage. When discovered, the wounds are usually badly infected and often require debridement. On occasion, involved areas have encompassed a 4- to 6-inch-wide patch of skin extending from the shoulders to the sacrum. Although at first these cases appear hopeless, they often respond to extensive debridement, fluids, and parenteral and local antibiotics. The addition of the enzymes, Elase (fibrinolysin and desoxyribonuclease) and Chymar (proteolytic enzymes, neomycin, and hydrocortisone), enhance healing. The exposed areas do not require skin grafting and in time are covered over by healed skin.

SEMEN COLLECTION AND EVALUATION

Semen evaluations are used routinely to evaluate new males about to enter the breeding program and at periodic intervals thereafter depending on an animal's reproductive performance.

Several methods for the collection of semen have been tried. A limited effort has been made to utilize prostate massage but this technique proved tedious and unsatisfactory. Internal (rectal) probes developed for use in domestic animals are effective when modified for use in the monkey. For the past 3 years, we have used a modified electroejaculation method described by Mastroianni and Manson (1963) utilizing a Grass stimulator (model SD5) (Valerio *et al.*, 1969b).

The electrodes are applied externally to the glans penis as follows: A strip of copper foil 2-cm wide is applied just above the base of the penis and held with a clamp electrode. The penis is moistened with saline to facilitate electrical contact with a second electrode held with a gloved hand against the glans near the frenulum of prepuce. Intermittent charges of 10-20 V are delivered at a frequency between 10-20 impulses/second for a duration of 30-50 msec, using a monophasic alternating current. If ejaculation does not occur in 1-2 minutes, the voltage is increased stepwise to 40 V; occasionally 40 V must be exceeded to accomplish ejaculation. Less than 10% of our males are refractory to this procedure. We have observed that repeated electroejaculations at 2- to 3-day intervals do not cause a refractory state.

Usually semen is collected from nonsedated monkeys using a monkey restraint chair (Foringer). Some males responded with ejaculation to electrostimulation after the use of Sernylan (phencyclidine hydrochloride), 0.6-0.8 mg/kg, intramuscularly.

The semen is liquid on ejaculation but within seconds a white to yellow coagulum (often referred to as a "plug") if formed. Liquefaction

begins within 5 minutes. Thirty minutes after emission about 30% of the semen is liquefied; it then continues at a slower rate but is never complete. The liquefied portion contains active spermatozoa.

A standard evaluation includes visual examination of semen for color and consistency, weight of ejaculate, cell count, motility gradation, count of morphologically abnormal sperm, and examination of a stained smear (eosin-nigrosin).

The tail of a spermatozoan is 70μ long and the head about 4μ wide. In the liquefied portion of the ejaculate, the number of spermatozoa ranges between 93 and 807 million/ml and the percentage of motile forms ranges between 30 and 98 (Mastroianni and Manson, 1963). All males with good reproductive performance records have sperm counts in excess of 200 million/ml (range 200-800 million/ml) and a few exceed 1 billion/ml. Semen from these same good breeder males usually show at least 70% motile spermatozoa.

ARTIFICIAL INSEMINATION

Artificial insemination techniques play a significant role in the production of domestic animals. Therefore, it is obvious to consider the application of this procedure to a simian breeding program. The methods of semen collection and artificial insemination have been recently adapted for use in *M. mulatta*. Mastroianni and Rosseau (1965) reported the recovery of fertilized ova after artificial insemination. However, most investigators have not been successful in impregnating monkeys by artificial insemination.

Within our breeding program in the last year, four female macaques have conceived from artificial insemination, the result of 35 attempts. All four, two *M. mulatta* and two *M. irus* females, have delivered normal, healthy babies.

The value of artificial insemination in a large simian breeding program, once the techniques are refined, is considerable. Semen from proved males could be used during the peak fall mating season. Then, if any substantial vestige of the seasonal influence persists, a relative shortages of males occurs and the male-female ratio of 1:8 in our colony becomes inadequate.

The storage of monkey semen is a problem even though it has been stored successfully for many domestic animals using gradual cooling and freezing. Egg yolk phosphate and egg yolk citrate have been used

as diluents. Boiled, pasteurized, homogenized milk and boiled, pasteurized, skim milk have been used as extenders. Glycerin is used to prolong viability and motility of spermatozoa. Antibiotics are frequently used to eliminate possible genital pathogens.

The semen utilized in our artificial insemination program is collected by the electroejaculation technique previously described under Semen Collection. It is collected directly into beakers warmed to body temperature. The liquified portion of the semen is inseminated into the vagina employing a tuberculin syringe. The coagulum is deposited into the vagina using thumb forceps and a speculum to dilate the vagina.

MATING

SEX PLAY AND COPULATORY BEHAVIOR

Males and females display many individual behavioral traits and responses, but grooming occurs when a receptive female is placed with a male in a breeding cage (Fig. 19). The male initiates sex play by stimulating the female with petting and by manipulation of the external genitalia. Copulation ensues and grooming may reoccur. The male may mount the female an exorbitant number of times. Evidence of a semen plug in the drop tray, or especially in the vagina, indicates that copulation and insemination have occurred.

Most dominant males will mount, copulate readily and frequently, and are seldom rebuffed by the inexperienced or unreceptive female. Males have been observed to protest fiercely and feign attack on the attendant if he attempts to remove the female from the cage. With some patience, the female can usually be isolated and taken from the cage.

While the breeding cage is large enough to accommodate two or more females and a male at one time, efforts to establish multiple breeding situations have proved unproductive. Less-dominant animals frequently incur injuries in such situations. Although ganghousing for breeding purposes is manageable, a social order must be established in any simian group and this requires time.

Our breeding operation employs single pairs of animals. The male's approach to the mating situation is distinctly better if the female is brought to his cage. If the opposite takes place, fighting frequently

Fig. 19. Male and female *M. mulatta* in breeding cage.

occurs. In either event (unless the male is well known to the handlers) the pair should be closely observed at the time they are put together to ensure that the female is not hostilely received. If they are compatible initially, they can be left with fair assurance that no serious fighting will occur.

BREEDING AND FERTILITY

Our breeding schedule is prepared once a week, based on the recorded menstrual cycle history of the females. All matings are planned in advance, using data of cycle lengths and previous mating

history. This also enables use of the same male that had previously impregnated the female scheduled for breeding. A female with a regular menstrual cycle length of 26-32 days is taken to the preselected male and mated for 72 hours, from the morning of day 11 to the morning of day 14. We have had pregnancies resulting from matings on days 7 to 11, as well as days 16 to 20 in females with short or long menstrual cycles. Data on our matings of *M. mulatta* for an entire year show a 15-18% conception rate, i.e., conceptions per total matings. Within the past 2 years, we have arranged about 5000 matings. An average of 3.5 matings were required for each female that conceived.

Many of the females in our colony are multiparous. One monkey has produced seven births within a 5-year period and several have produced at least five births.

The seasonal breeding of *M. mulatta*, as well as other simians, has long been an established fact and is in the fall and early winter months. While no attempt is made here to explain the phenomenon, it could be attributed to a number of environmental, ecological, and physiological factors. The fall and early winter months are the prime breeding period for recently imported monkeys, but this becomes less evident in animals that remain in the colony over a long period. Since each year the colony acquires newly imported pregnant monkeys, which deliver in the spring and are not rebred until the following autumn, the natural breeding season is artificially maintained for a while. Under laboratory conditions, the female *M. mulatta* has a menstrual cycle through all months of the year.

In a study of the seasonal phenomenon, our results were somewhat biased by the fact that most simians have been in the colony only 6-12 months. This was mainly responsible for the 73% of conceptions occurring in the months of September through January and reflecting the natural breeding season. The lowest number of conceptions (eight) was in April and July but there was no significant difference from March through July. Although the study showed the persistence of a seasonal breeding pattern, it is somewhat artificial because of the relatively short time in the laboratory for most of the females.

Marked attenuation of seasonal breeding in the laboratory is shown in Table VI, a summary of births from 1963 through 1966. There is an increasingly even distribution of births throughout all months of the year as the colony became more adapted to a laboratory environment.

Table VI

Monthly Distribution of Total Number of *M. mulatta* Births

Month	1963		1964		1965		1966	
	Bred in nature	Bred in lab.	Bred in nature	Bred in lab.	Bred in nature	Bred in lab.	Bred in nature	Bred in lab.
January	1	0	0	6	2	3	4	9
February	17	0	3	8	46	1	21	17
March	22	2	12	6	101	11	11	42
April	75	2	49	16	35	6	18	43
May	11	0	26	15	24	14	31	48
June	2	5	11	15	7	18	7	39
July	1	2	4	7	3	12	1	28
August	3	2	0	9	1	9	0	15
September	1	2	0	8	0	10	0	7
October	0	3	0	1	0	5	0	7
November	0	7	0	2	0	7	0	13
December	0	6	0	6	1	2	0	16

INFERTILITY

Infertility among simians is a problem. Breeding records are reviewed and after considering such factors as arrival date, times of year mated, and history of male mates, we consider a female infertile after 10 to 12 matings without conception. There are many possible causes: anatomically abnormal reproductive organs, genital infections, malnutrition, and hormonal imbalance with concomitant physiological malfunction.

The etiology of oligomenorrhea, or amenorrhea, of females that have previously demonstrated reproductive abilities is unknown. Some are the result of uterine infections and others are likely the result of primary hypofunction or dysfunction of the ovarian-pituitary-hypothalamic complex.

Then there are females, previously fertile, that have had regular menstrual cycles but persistently fail to conceive. Animals representative of this group were selected for a study of hormonal imbalance which is still in progress (Valerio and Courtney, 1968).

Clomiphene citrate (Wm. S. Merrell) is a synthetic nonsteroid com-

pound. Although presently an investigational drug, it has been found to be effective in stimulating ovulation in previously infertile women. Forty-seven infertile, but otherwise normal, healthy and mature female monkeys were selected for a study. A summary of the 6-month observation is as follows. Females were treated with 3 mg/kg of clomiphene citrate for 3 consecutive days at monthly intervals depending on the type of menstrual cycle observed. The results showed 15 of the 27 treated females became pregnant after an average of 2.3 monthly treatments. This was significantly different from the 3 out of 20 pregnancies among the infertile, nontreated controls. Further clinical investigation of other routes of administration and other dosages of clomiphene citrate are being conducted. We have demonstrated that clomiphene citrate is helpful in regulating the cycle of oligomenorrheic monkeys and inducing the cycle of amenorrheic monkeys. Since multiple births have been reported frequently in women on clomiphene therapy, we are particularly interested in the dosage necessary to create superovulation with the compound in monkeys.

Microbial causes of infertility have had very limited investigation. Common isolates from vaginal discharges have been *Escherichia coli*, *Proteus vulgaris*, and *Staphylococcus aureus* (coagulase positive).

Vibrio fetus was isolated from the abdominal fluid of one adult female *M. mulatta* showing clinical signs of lethargy, weight loss, and slight chronic diarrhea. This clinical picture (and its apparent frequency of occurrence) is very different from the well-established havoc caused by vibriosis to the reproductive process in cattle and sheep. Our finding is similar to that found in monkeys at the National Center for Primate Biology (Good, personal communication). As yet, the implications of vibriosis in monkeys are poorly defined and in this respect may parallel the consequences of *V. fetus* in humans (King, 1957; Hood and Todd, 1960; Kahler and Sheldon, 1960).

Many aspects of infertility are attributable to the female, but fertility problems of the male should not be overlooked. Certainly, the cause for the well-noted seasonal breeding variability should be considered. It was reported that in *M. mulatta* in nature, there was a lack of spermatogenesis for 7 months of the year, as determined by testicular biopsy (Conaway and Sade, 1965). The seasonal variation of natural fertility, the increased testicular weight, and increased semen volume with corresponding higher sperm counts during the natural breeding season should not be overlooked (Sade, 1964). The males in our laboratories have produced impregnations in all months of the year. In

only a few males has a seasonal influence on reproductive performance in the laboratory been observed.

DETECTION OF PREGNANCY

INTRARECTAL PALPATION OF THE UTERUS

All females in breeding are given a digital intrarectal palpation of the uterus for confirmation of pregnancy at 25-30 days postbreeding (Hartman, 1932). The animal is held by an attendant in a one-handed arm restraint position while, with his free hand, he holds the head. Occasionally, a second attendant holds the lower limbs. A veterinarian or technician, wearing a disposable glove, inserts the middle finger into the rectum and completes the examination of the uterus using the other hand of counter pressure against the lower abdomen. The nonpregnant uterus is approximately 7.5 × 15 mm, although it varies considerably with the age of the animal. The ovaries and cervix are palpated by this manner as well. At about the time of implantation bleeding (15-21 days) subtle changes of the uterus can be noted and usually it is twice the preconception size and firmer. Trying to detect pregnancies so early can often be deceiving unless the preconception uterine size has been well observed. However, by 28-30 days, the uterine size is 30-40 mm and there is an elastic tone and softening of the wall of the uterus. The size of the pregnant uterus is correlated with the stage of gestation as follows: 28-30 days (3 × 4 cm); 45-50 days (5 × 6 cm); 65-80 days (7 × 8 cm). By about 85 days, the fetus can be palpated abdominally. Hartman (1932, 1933) described the techniques of palpating the uterus and ovaries and the correlation of age and texture with various stages of gestation.

BIOLOGICAL TEST FOR PREGNANCY

The only biological test for pregnancy used by us is a modification of the Ascheim-Zondek method which depends upon detecting the presence of chorionic gonadotropin in the serum of pregnant *M. mulatta*. Using the uterine weight of mice as a parameter, the protein hormone of the simian placenta can be detected as early as the eigh-

teenth day of pregnancy with reliability. The test is not used routinely, but only when it is essential or desirable to confirm a pregnancy earlier than 25 days of gestation.

Eighteen days after breeding, 10 ml of blood are collected from the female monkey for the test, and serum is removed and refrigerated (frozen, in the event the serum is not used that day). Immature female mice, 15-20 days of age and weighing between 7 and 9 gms are used. Mice less than 15 days old do not give a complete response to chorionic gonadotropin while mice over 21 days produce their own hormone and may yield false positives. Three mice are each injected subcutaneously with 0.5 ml serum daily for 3 days. The mice are examined postmortem on the fourth day and the uterine horns are dissected out, blotted on paper toweling, and weighed. A 100% increase in uterine weight over that of untreated controls is considered a positive response. The controls are divided into two groups (Tullner and Hertz, 1966): (1) positive controls – mice injected with chorionic gonadotropin consisting of total doses of 0.2, 0.1, and 0.05 I.U. The use of positive controls is important to determine whether or not the uteri of test animals are responsive to injected hormone as well as for quantitative purposes since other factors can affect the end organ response; (2) negative control – three mice are injected with sterile saline.

PREGNANCY

Early signs of conception are usually associated with implantation bleeding, which is referred to as the "placental sign." While others have stated that all simian pregnancies are associated with implantation bleeding, we have found 3-5% of females do not show the phenomenon. Implantation bleeding begins between 15 and 22 days after conception and usually lasts from 10 to 20 days. Although it is nearly diagnostic of conception, it usually begins about the time the menstrual hemorrhage would. It is thus undistinguishable from menses until the bleeding is protracted.

Accurate breeding dates are essential for known gestational stages. The average gestational age at birth for *M. mulatta* is 165 days, which, based on a 72-hour mating, has an inherent error of plus 1 day or minus 3 days since spermatozoa are viable up to 48 hours. As pregnancy advances toward term, abdominal palpation becomes valuable. While

we rarely have been able to auscultate the fetal heart beat, fetal anatomical structures are readily palpable through the abdominal and uterine walls.

The management of pregnant monkeys requires special attention. Once a pregnancy is established, the female's permanent cage is identified with a red tag signifying to the attendant that it is no longer subject to the daily vaginal swab. Instead, he observes the female closely for further implantation bleeding which is recorded and reported daily. If observable implantation bleeding continues (as seen in the drop trays or on the perivulvular area) longer than 10 days, hematinic therapy is instituted. Hematinics are administered on alternate days for a total period of 10 days. They consist of NOnemic (iron dextran), liver B_{12}, and folic acid alternating with Coferrin and Poly-B vitamins. All of the hematinics are administered in 1.0-ml amounts intramuscularly. If implantation bleeding is prolonged beyond 15 days, threatened abortion is suspected and progesterone therapy is instituted as well. Delalutin (125 mg) is given every 3 days for a maximum of three injections. This therapy has appeared clinically beneficial although no controlled efficacy study has been undertaken by us.

There are many routine examinations that involve handling of pregnant females but care is taken to do no more than is essential. Two weeks after diagnosis, the pregnancy is reconfirmed as a routine procedure by intrarectal palpation. This is considered necessary because 35% of all abortions occur in the first 2 months of pregnancy. Thereafter, the females receive a monthly palpation of the uterus to determine the status of the conception throughout pregnancy. A pregnant female is a healthy normal animal, eats well, is bright, alert, and maintains her usual aggressive behavior until near term when she may develop a more subdued disposition. Blood samples are routinely tested for a complete cell count and sedimentation rate. Physical examinations are made on a bimonthly basis. The hematological results have been very valuable indices of prepartum general maternal health as well as threatened abortion. This is discussed in detail in Chapter VI.

Pregnant females are weighed monthly throughout gestation. While they show little weight increase for the first 80 days of gestation, they usually gain between 0.5 and 1.0 kg during pregnancy. Once weekly for the last 3 weeks of pregnancy all females are palpated to determine fetal position *in utero*. This is vitally important since fetuses in

posterior presentation (breech) usually die if natural delivery is allowed.

DELIVERY

NATURAL DELIVERIES

The infant is carried high in the abdominal cavity until near term when it drops into the pelvic position. Our observations have been that cervical and vaginal dilation can occur in a matter of hours just prior to delivery. Blood and amniotic fluid may be noticed in the drop pan immediately preceding parturition which is of relatively short duration. Following an uncomplicated delivery the mother will chew the umbilical cord, cut it with her teeth, and eat the placenta. The mother will immediately clean the baby and clasp it to her breast. Normally, baby monkeys are delivered by natural means without assistance and usually between the hours of 10 P.M. and 4 A.M. Only 1% of our infants are born in the daylight hours.

CESAREAN SECTION

Cesarean sections are performed (1) if the fetus is in a posterior position 5-7 days prior to expected delivery date; (2) if delivery is more than 10 days late; (3) if a suspected dead conceptus fails to be delivered naturally by the time of due date; (4) if the female shows signs of severe toxemia; (5) if a moribund imported female is estimated to be within 3-4 weeks of term; or (6) if a research program requires premature infants or samples of amniotic fluid and placenta (in which case, cesarean sections are performed at about 163-days gestation).

Although imported pregnant mothers are poor surgical risks, 40 mothers and 47 babies survived 91 cesarean sections performed on animals of this group. In 13 cases, the surgery was performed to remove autolyzed conceptuses. Among laboratory-bred animals, overdue by 10 days or with complicated deliveries, 53 cesarean sections resulted in survival of 52 mothers and 48 infants.

Delivery by cesarean section does not impair the animal's health or potential ability for subsequent natural delivery. Many of the colony's

multiparous females have delivered alternately by natural and surgical means.

For a description of the cesarean section surgical procedure, see p. 115.

BIRTH ABNORMALITIES

ABORTIONS AND STILLBIRTHS

Approximately 15% of females that conceive can be expected to abort or produce a stillbirth, but causes of abortions and stillbirths are unknown. A number of pathogens (i.e., *Toxoplasma, Vibrio sp., S. aureus*) have been implicated from samples of abortuses or maternal tissues, but no causative relationship to the abortions has been established. Premature separation of the placenta and placental insufficiency have been observed occasionally. These conditions undoubtedly cause abortions and stillbirths during all stages of gestation.

With imported pregnant monkeys we anticipate that 50–75% of them will abort or produce stillbirths. With extensive care the degree of fetal wastage can be minimized but rarely brought below a 30% abortion incidence. Fetuses die in transit since abortion of an autolyzed specimen often occurs shortly after arrival. The nutritional and disease states of the mothers probably predispose the outcome precipitated by the stress of transit.

A survey of 42 imported pregnant *M. mulatta* (Table VII) showed the grossly abnormal blood counts that can be encountered when they arrive in the colony. In particular, the average hematocrit is lower and the white blood cell count is higher than those found in laboratory-bred pregnant monkeys. A vast majority of these animals aborted within the first 2 weeks of arrival and many others had pneumonia and diarrhea.

Comparing the number of live births from laboratory-bred versus imported pregnant monkeys, it can be seen from Table VIII that most imported pregnant monkeys abort or produce stillborns, whereas the incidence in laboratory-bred monkeys is very low. The significantly higher number of deaths observed for female fetuses than for male fetuses among the imported pregnants is inexplicable.

If the first birth is nonviable, whether the mother was a newly im-

Table VII
Hematological Values of 42 Pregnant *M. mulatta* on Day of Arrival

	Average	Standard error
Hematocrit (%)	39.4	±.70
Hemoglobin (gm%)	12.8	±.22
Sedimentation rate (mm per hour)	16.9	±2.43
White blood cell count (per mm^3)	15,500	±1,360
Myelocytes (%)	0.19	±0.09
Juveniles (%)	1.6	±0.35
Bands (%)	6.1	±1.14
Segmented neutrophils (%)	55.5	±3.04
Lymphocytes (%)	30.2	±2.92
Monocytes (%)	3.98	±0.33
Eosinophils (%)	1.02	±0.24
Basophils (%)	0.5	±0.08

Table VIII
Comparison of Breeding Status on Birth Viability of *M. mulatta*

	Mother imported pregnant			Mother laboratory-bred		
Births	Live	Dead	Total	Live	Dead	Total
Male	137	84	221	277	18	295
Female	134	204	338	259	24	283
Sex unknown	–	102	102	–	38	38
Total	271	390	661	536	80	616

ported pregnant or a laboratory-bred monkey, the incidence of abortions and stillbirths for all subsequent pregnancies is about 15%. Thus, even though an imported pregnant animal aborts initially, it is subsequently as capable of producing live babies under laboratory conditions as any other female.

ANOMALIES

All babies are examined grossly for malformations, general state of health, growth, and development. Only one anomaly, a clubfoot, has been observed in 900 births from laboratory-bred monkeys.

However, a number of anomalies were seen in the babies of imported pregnant monkeys. Of note is one fetus that displayed bilateral

amelia of the arms and bilateral hemimelia of the legs (Fig. 20). The anomalies of this specimen are quite typical of those produced by thalidomide both in human beings and monkeys.

We have also observed one specimen with syndactylia, one with polydactylia, and three with supernumerary nipples.

A few anomalies have been seen in imported adult monkeys. Among males there was one each of the following: oligodactylia, lobster-claw hands, facial hemangioma, and cryptorchid testis. In the females, supernumerary nipples and a constricture of the vagina have been seen.

TWINNING

One imported pregnant *M. mulatta* gave birth to twins. When rebred

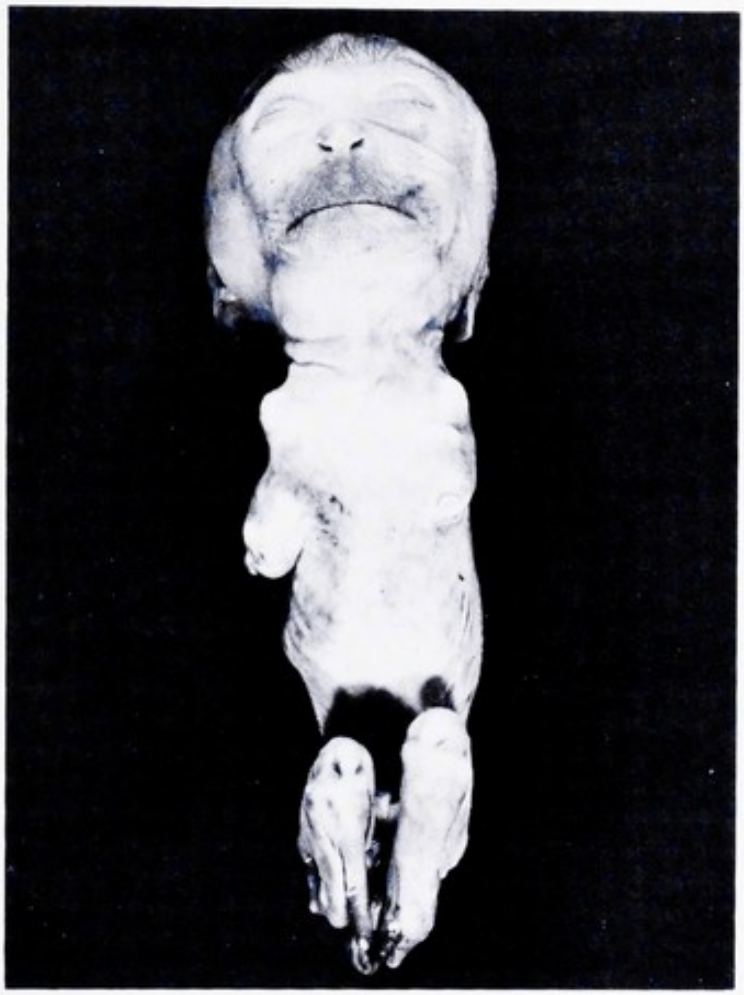

Fig. 20. *Macaca mulatta* fetus with bilateral amelia of arms and bilateral hemimelia of legs.

in the laboratory she produced another set of twins. Recently, another laboratory-bred *M. mulatta* produced fraternal twins, both males. These three cases of twinning are the only ones observed in our colony (Courtney and Valerio 1968; Schulz 1956).

SEPARATION OF BABIES FROM MOTHERS

A mother naturally delivered of a baby is allowed to spend an hour or so cleaning the infant, severing the umbilical cord, and disposing of the placenta. Most females are possessive and handle the newborn protectively. Usually within 1–3 hours from birth, the infant is separated from the mother quickly and gently by two animal attendants, or by one attendant with the use of a tranquilizer. While one must be aware of potential hazards, rarely has a mother been disturbed enough to injure her infant. Trained, experienced attendants must exercise patience and gentleness to separate baby from mother. Often the latter is tranquilized with an injection of Tranvet (propiopromazine hydrochloride, 2 mg/kg, intramuscularly). After waiting 10–15 minutes, the baby can be removed easily. In the initial step of the procedure the mother and infant are gently restrained to the forward part of the cage in such a manner that the mother faces one side. The infant usually is clasped to the mother's abdomen and is now located near the door opening. While one attendant raises the door slightly, another reaches in and restrains the mother's head; the first attendant then removes the baby. The process of separation has been performed with uniform success and with only rare injury to mother or infant.

POSTPARTUM CARE OF MOTHERS

Following parturition, the placenta is usually passed within an hour or two. The placenta in *M. mulatta* is bidiscoidal and weighs from 130 to 170 gms. If females fail to pass the placenta within 24–72 hours after parturition, surgical intervention is indicated in the form of a dilatation and curettage operation (see p. 119). A female with a retained placenta usually becomes toxemic and commonly develops secondary systemic complications.

Postpartum routine treatments include a 7-day course of hematinics, as well as an antibiotic (Longicil) which is given prophylactically after delivery and repeated in 3 days at a dose of 30,000 units/kg to prevent uterine infection, especially of *S. aureus*.

Any uterine infection that develops is identified bacteriologically and appropriate antibiotic therapy is instituted based on sensitivity tests. All females are subjected to postpartum hematological examination at 24 hours, 3 weeks, and 8 weeks.

Most females resume menstruation within the first 45 days postpartum. All females are rested for a minimum of 2 months before reintroduction into the breeding program, although for some this is extended to 3 months depending on physical state, uterine condition, natural resumption of the menstrual cycle, and hematological results. All females receive vaginal swabs starting with day 1 postpartum. At 2 months after parturition, the females receive physical examinations which include palpation of the uterus and observation for uterine or vaginal discharges. Provided everything is found within normal limits, the female is released for further breeding.

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VI SIMIAN MEDICINE

Simian medicine is little different from the practice of internal medicine of other animals in that it must be largely based on an understanding of pathology. In establishing a diagnosis in monkeys, the clinician relies more on data provided by the various diagnostic services (radiology, hematology, bacteriology, parasitology, pathology) than on physical findings. Clearly, the very nature of the monkey makes physical examination somewhat restricted. Physical signs of disease are changes in temperament and appearance, departures from the normal activity in a cage, feeding abnormalities, and other disturbances specifically related to functional impairment of organ systems.

When large numbers of simians are imported into an existing colony the problem of zoonoses is constantly present and great losses are a concomitant problem. So too, if large numbers of Old World and New World monkeys are kept together in one building, even though individually caged, and newly imported animals are regularly added, there must be alertness to the dangers of transmission of disease. Diseases pass from simian to simian, from simian to man, and manifestly also from man to simian—although the latter may not be easy to prove beyond doubt. Such diseases include: tuberculosis; leptospirosis; streptococcal, staphylococcol, meningococcal, and pneumococcal infections; and dysentery (mainly shigellosis and salmonellosis).

BACKGROUND COMMENTS

More is known about pathology than about clinical aspects of simian diseases. In accordance with the stated aims of this guide, some indications of literature on diseases of the *M. mulatta* may help those new to the field.

As a source of reference for the anatomy and physiology of simians, see the classic *Bibliographia Primatologica* compiled by Ruch (1941). For data on the normal anatomy of the *M. mulatta*, see Hartman and Straus (1933), now reprinted (1961). Between 1940 and 1959, Ruch collected data and edited an invaluable book on *Diseases of Laboratory Primates* which contains a vast bibliography and excellent illustrations. Patricia O'Connor Halloran (1955) brought together thousands of references in *A Bibliography of References to Diseases in Wild Mammals and Birds* which includes more than 60 pages of references on simian conditions. Three of these publications are unfortunately out of print and obtainable only through libraries, but workers anxious to trace original works must refer to them. The reports from the London Zoological Gardens, dating back more than 70 years, contain much information on simian diseases, (first by H. H. Scott; later by A. E. Hamerton, R. E. Rewell, W. C. Osman Hill; and recently by R. N. Twistleton-Wykeham-Fiennes). Also valuable in this respect are the reports from the Penrose Laboratory, Philadelphia Zoological Gardens, (first by C. Y. White, then by H. Fox, and currently by H. L. Ratcliffe). The long experience of H. Fox culminated in 1924 with the production of *Disease in Captive Wild Mammals and Birds*, unobtainable except from libraries but still an important source of information.

For readers of German, there is a wealth of data on simian diseases in many chapters of the two volumes of Cohrs, Jaffé, and Meessen (1958) which cover the range of so-called laboratory animals including simians, other mammals, birds, amphibia, fish, and reptiles. Without separate chapters on simian diseases, the work must be scanned for topics dealt with either on an etiological basis (Vol. II) or as a special pathology of organ systems (Vol. I).

Twistleton-Wykeham-Fiennes (1967) produced an incomparable treatise on *Zoonoses of Primates. The Epidemiology and Ecology of Simian Diseases in Relation to Man,* in which the whole range of infective agents causing disease are discussed relative to those transmissible between all primates.

Several original pathological studies on fairly large numbers of simians include: Fairbrother and Hurst (1932), 600 animals; Habermann and Williams (1957), 707 *M. mulatta* and *M. philippinensis*; Kennard and Willner (1941), 216 *M. mulatta*; Kennard (1941), 246 monkeys; Sauer and Fegley (1960), 500 monkeys; Weston (1965); Jungherr (1965). Good descriptive details on the pathology, including neuropathology, of experimental infections produced by viruses and rickett-

sia are in van Rooyen and Rhodes (1966) and in Rivers (1965). For papers on experimental work on bacterial and viral diseases in monkeys, see Wilson and Miles (1961). The indices of the *Veterinary Bulletin* should always be scanned for work on simians. The *Bulletin* (founded in 1928) each year searches 4000-5000 journals and books for publications relating to veterinary and comparative pathology at large. The 70 volumes of the *Journal of Pathology and Bacteriology* (1892-1962) include a number of articles on natural and experimental diseases of monkeys (see Oliver and Boyd, Edinburgh, 1965 for Index).

Parasitic infestations (alimentary) are dealt with on pp. 40-41. Standard medical or veterinary texts on helminthic and protozoan parasites do not include many references to monkeys. Twistleton-Wykeham-Fiennes (1967) gives a very complete list as does Graham (1960); (see also Mendheim in Cohrs *et al.*, 1958, pp. 201-211). In *M. mulatta*, the most important helminthic parasites are alimentary ones which can cause devastating ill health. However, largely because infestations involve several species of worms, it is impossible to ascertain the extent of effect of particular species.

HEMATOLOGICAL OBSERVATIONS*

The extensive use of the *M. mulatta* in biological research has necessitated frequent reference to normal hematological data, especially in the evaluation of the clinical status of the research animal. Much of the data presently available (Schalm, 1965; Krise and Wald, 1958; Krise, 1960; King and Gargus, 1964) provide a limited amount of information, or have been derived from small groups of animals.†

One of the more serious voids is the lack of defined data associated with the period of conditioning or the types of housing employed. In the absence of well-defined normal blood values, the investigator and the clinician are frequently unable to specify with any certainty just what may constitute a "normal" animal. A further complication is the wide variation in so-called "normal" values found in most wild animal species, including simians.

One of the more difficult problems involved in observing simians

*Contributed by John W. Switzer and Bonnie Rininger.
†See also Huser (1969).

(and other wild animals) is the contradiction of the animal's apparently normal physical appearance with grossly abnormal laboratory findings. For example, simians with severe anemias, with as low as a 10% hematocrit, fail to manifest physically the severity of their condition. The clinician must frequently rely upon laboratory data for a more meaningful evaluation of the animal's status because often even the most subtle clinical signs are accompanied by severely altered blood values.

The hematology laboratory in a large simian colony offers a distinct advantage in the routine care of the animals. Hematological examinations can detect hidden disease and measure the degree of severity. In routine care of breeding animals, hematology can identify subnormal animals for appropriate therapy. Anemia is frequent among simians and may be treated when diagnosis is established. A large proportion of anemias are often associated with chronic bacterial disease and generally respond when the primary disease is determined and treated. Iron deficiencies are encountered and are usually accompanied by a history of chronic blood loss associated with diarrhea, parturition, lacerations, or surgery. Undoubtedly, malnutrition also plays a role in the etiology of anemia.

A hematocrit level of less than 37% is a significant indication of anemia in the *M. mulatta*. Animals with values below this level should undergo additional diagnostic procedures to determine the cause of anemia. White counts above 15,000/mm^3, increased sedimentation rate, increased band neutrophils, and the presence of Doehle's bodies in the neutrophils are a definite and significant indication of disease. Often the cause cannot be specifically determined. However, the hemogram can be expected to return to normal in most cases after a short course of antianemic or antibiotic therapy.

TECHNIQUES

Blood is obtained by femoral venipuncture and placed in either 5-ml or 3-ml Vacutainers containing dipotassium versenate (EDTA). The 3-ml Vacutainer has been very satisfactory and is particularly suitable for obtaining 1-ml amounts of blood from baby monkeys. It must be emphasized that excessive EDTA causes erythrocyte shrinkage; therefore, the size of tube used and the amount of EDTA contained should conform to the quantity of blood withdrawn. The general recommendation of 2.5 mg of EDTA per milliliter of whole blood should be ad-

hered to fairly closely since excessive EDTA causes significant reductions of hematocrit and mean cell volume (MCV) values. Hemolysis and clotting of the sample are obviously to be avoided. Partial clotting results in lowered leukocyte and platelet counts and decreased hemoglobin levels. In certain situations – particularly in the case of newborn animals in isolators – the Unopette may be used to collect blood for complete blood counts although our experience has been that clotting in the capillary collection tube of the Unopette occurs frequently and can lead to gross laboratory error. Consequently, many samples must be drawn to obtain valid results. In our experience, venipuncture is preferable to the use of Unopettes. Venipuncture offers the additional advantage of permitting replicate determinations from a single sample. Venipuncture is accomplished using a femoral vein. (Whereas the saphenous or cephalic is used for intravenous administration, these tend to collapse upon blood withdrawal while the femoral does not.) Venipuncture is used for all complete blood counts, but not when repeated daily specimens are required for some particular research study. For single test determinations, e.g., microhematocrit or WBC differential, heel puncture is performed using a lancet (Fig. 21).

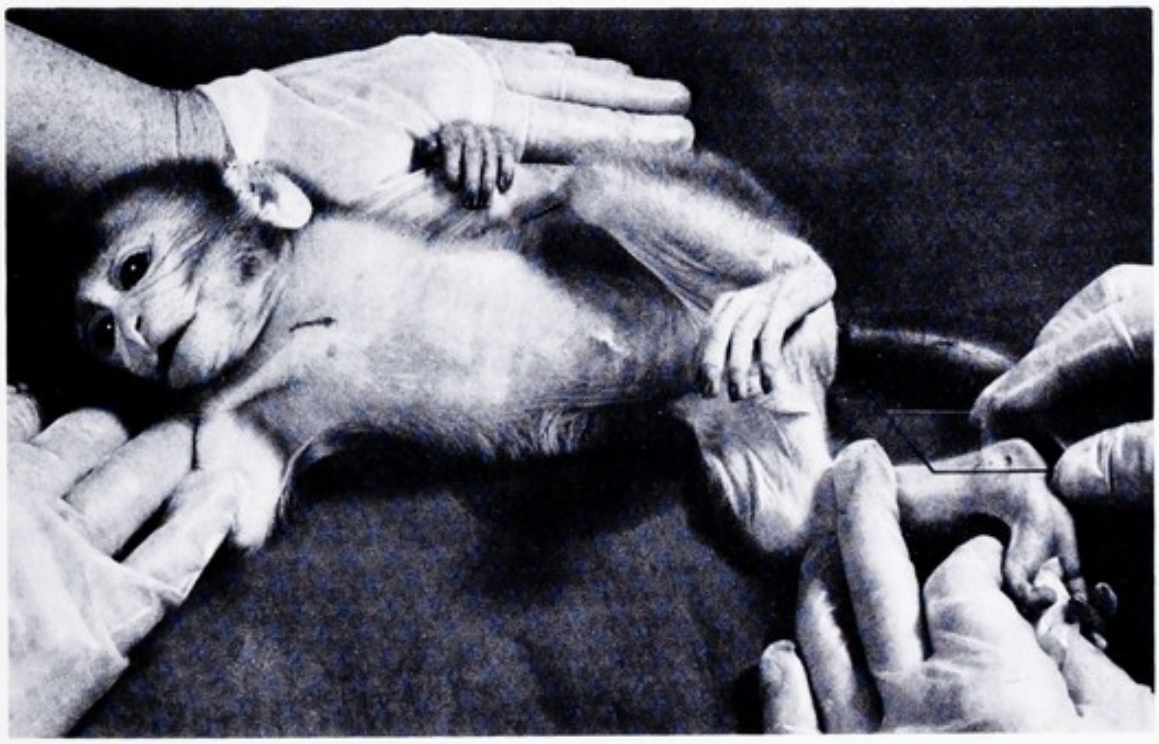

Fig. 21. Heel puncture for blood smear of infant.

Standard methods are used to perform the blood counts. The Coulter counter, Model B, is used for red and white cell counts. Autodiluters are used to make the appropriate dilutions for red and white cells and hemoglobin. Microhematocrit is accomplished using 1 × 75 mm capillary tubes and the International centrifuge (Model MB). Hematocrits are read with an International hematocrit reader (Model-CR). Disposable Wintrobe tubes and a Sedi-Rack are used for the sedimentation determination which is performed within 1 hour after withdrawal of blood from the animal. Reticulocytes are stained supravitally in a solution containing new methylene blue (0.25%) and rhodamine B (0.10%) in 0.38 *M* sodium nitrate and 0.85% sodium chloride with 100 ppm sodium azide added as preservative. Two to three drops of blood are added to a small tube containing two drops of the reticulocyte stain and incubated 5 minutes at 37°C. Slides are prepared and 1000 red cells counted to obtain the percentage of reticulocytes. Platelet counts are performed by the method of Brecher and Cronkite (1950).

Slides for differential leukocyte counts are stained in modified Wright's stain (Reich, 1954) using the dip-jar technique (Schalm, 1965) with slide holders containing up to 30 slides. Differential leukocyte counts are performed by counting 100 cells under oil immersion. Because of the variability of the morphological appearance of simian leukocytes – particularly lymphocytes and monocytes – considerable training and experience are required.

HEMATOLOGICAL VALUES*

This section presents hematological data derived from adult *M. mulatta* that have been maintained for long periods of time under our specified environmental and nutritional conditions, and from infants hand-reared from birth under isolation.

The results of determinations on conditioned adult animals are presented in Table IX.

Adult animals used to establish normal values were housed in the laboratory for periods of 6 months to 3 years. All animals were free of clinical diseases when the study began. The females were nonpregnant and more than 60 days postpartum. Note that the hematocrit of males is significantly higher than that of females. Note also the wide standard deviations from the means which are characteristic of *M. mulatta*. Because of this high standard deviation, elimination of ani-

*Erythrocytic counts have not been included in the tables because of insufficient data. MCV values for adult *M. mulatta* are between 68 and 80 μ^3.

Table IX

Hematological Values for Normal Adult *M. mulatta*

	Male ($n = 104$)			Female ($n = 77$)		
	Average	Range[a]	S.E.[b]	Average	Range[a]	S.E.[b]
Hematocrit (%)	44.8	37.7-51.9	0.347	41.8	36.4-47.1	0.307
Hemoglobin (gm%)	14.4	12.0-16.8	0.119	13.5	11.7-15.3	0.104
Total white blood cells (per mm^3)	10,290	4,712-15,868	2.75	10,253	4,759-15,747	3.13
Differential						
Juveniles	0.01	0-0.19	0.009	0.03	0-0.47	0.0261
Percent per mm^3	1.4	0-30.2	1.40	3.5	0-65	3.47
Bands	0.13	0-0.97	0.041	0.09	0-0.77	0.0387
Percent per mm^3	15.0	0-116	4.91	10.2	0-89	4.48
Segmented neutrophils	32.9	3.6-62.3	1.44	38.7	14.7-62.5	1.36
Percent per mm^3	3,448	170-7,804	213.8	3,988	585-7,389	193.9
Lymphocytes	58.0	29.0-87.0	1.42	52.1	29.6-74.6	1.281
Percent per mm^3	5,718	1,619-9,815	201.0	5,327	1,639-9,015	210.2
Monocytes	4.2	0-9.0	0.235	3.2	0-8.4	0.30
Percent per mm^3	428	0-983	27.32	323	0-885	31.63
Eosinophils	4.1	0-10.1	0.299	5.4	0-12.2	0.387
Percent per mm^3	420	0-1,142	36.06	548	0-1,292	42.4
Basophils	0.4	0-1.7	0.0616	0.5	0-1.9	0.082
Percent per mm^3	42	0-190	7.21	48	0-204	8.94
Unclassified (%)	0.05	0-0.5	0.023	0.05	0-0.5	0.0261
Sedimentation rate (mm/hr)	0.51	0-3.0	0.12	0.51	0-2.5	0.11

[a]Two standard deviations.

[b]Standard error.

mals failing to fall within the two-standard-deviation (2 S.D.) range serves to eliminate only the most severe deviations from normal. Therefore, for the purpose of group analysis, it appears that the use of standard error (S.E.) is far more useful in detecting differences in experimental groups.

The effects of pregnancy and the postpartum state on the blood count are presented in Table X. All animals selected for this study were bred in the colony on known breeding dates and all delivered a live baby at term. Several animals were deleted from the postpartum study because of obvious metritis or postpartum complications.

Note that the average hematocrit in the initial 30- to 60-day period is lower than during the remainder of the pregnant state. Studies presently underway indicate that this reduced value betrays implantation bleeding which occurs around 15-20 days after conception.

The most significant change in the blood count is seen in the period immediately following parturition at which time there is a 5% decrease in the hematocrit reading. This marked decrease reflects the blood loss during parturition and, from these results, appears to be of a greater magnitude than previously suspected. Other changes occurring at the time of parturition include a fivefold rise in the sedimentation rate, a slight rise in total neutrophils, and a marked decrease in eosinophils. Several weeks following parturition, there is a twofold increase of total eosinophils and a return of sedimentation rate and hematocrit to normal levels. Within 60 days postpartum, the hemogram approaches normal levels with the exception of an elevated eosinophil count.

In a later study of the immediate postpartum period with samples taken at 24, 48, 72, and 96 hours and 7 days after delivery, it has been observed that there is a continued decline in the hematocrit which often does not reach a minimal value until 72-96 hours after birth. The degree of decline is thought to be proportional to the amount of blood loss at time of delivery. From the preliminary results of this present study, it appears that blood samples taken before 72-96 hours postpartum may not reveal the true status of the animal or the full extent of blood loss at delivery. As one might expect, hematological values do not return to normal until blood volume has been restored.

Bone marrow values for adult *M. mulatta* are presented in Table XI. The values were determined on selected healthy animals existing in the colony from 9 months to 3 years (Switzer, 1967a). Bone marrow aspiration samples were obtained from the tuber ischii. The complete details of the procedure are given by Switzer (1967b).

TABLE X

Hematological Values for Adult Female *M. mulatta*, Pre- and Postpartum[a]

	34 Animals, 138–111 days prepartum	38 Animals, 110–88 days prepartum	47 Animals, 87–60 days prepartum	48 Animals, 69–32 days prepartum	48 Animals, 31–1 days prepartum	48 Animals, 1–60 hours postpartum	24 Animals, 14–21 days postpartum	21 Animals, 22–28 days postpartum	46 Animals, 46–77 days postpartum
Hematocrit (%)	40.5 ± 0.52	42.4 ± 0.44	43.1 ± 0.46	42.5 ± 0.93	41.5 ± 0.50	35.6 ± 0.95	41.6 ± 0.57	42.1 ± 0.54	42.5 ± 0.49
Hemoglobin (gm %)	12.7 ± 0.19	13.3 ± 0.15	13.6 ± 0.15	13.6 ± 0.15	13.2 ± 0.16	11.5 ± 0.21	13.5 ± 0.17	13.6 ± 0.19	13.8 ± 0.16
Sedimentation rate (mm/hr)	1.8 ± 0.34	1.5 ± 0.28	1.1 ± 0.16	2.2 ± 0.29	5.9 ± 0.77	21.0 ± 1.98	0.8 ± 0.22	0.3 ± 0.13	0.3 ± 0.14
Total white blood cells (per mm^3)	10,591 ± 512	10,760 ± 555	11,338 ± 557	10,506 ± 455	10,185 ± 354	10,994 ± 789	10,791 ± 725	11,448 ± 810	11,396 ± 448
Segmented neutrophils (per mm^3)	4,814 ± 366	4,480 ± 287	5,697 ± 450	5,401 ± 363	5,068 ± 226	6,853 ± 689	3,073 ± 274	3,975 ± 600	3,435 ± 314
Lymphocytes (per mm^3)	4,603 ± 282	5,049 ± 346	4,353 ± 279	3,763 ± 417	3,798 ± 252	3,166 ± 228	5,781 ± 484	5,587 ± 327	6,509 ± 324
Monocytes (per mm^3)	481 ± 54	469 ± 47	489 ± 49	465 ± 41	542 ± 40	541 ± 69	457 ± 61	493 ± 58	366 ± 50
Eosinophils (per mm^3)	643 ± 80	694 ± 74	713 ± 61	775 ± 74	676 ± 81	295 ± 39	1,357 ± 139	1,302 ± 138	1,026 ± 102
Basophils (per mm^3)	48 ± 26	57 ± 15	61 ± 14	81 ± 12	73 ± 16	62 ± 23	81 ± 21	77 ± 25	52 ± 11

[a]Values given represent averages, plus or minus standard error.

Table XI
Bone Marrow Values in Adult *M. mulatta*

	Female (%)	Male (%)	Average (%)	S.D.	Range (%)
Erythrocytic cells					
Rubriblasts	0.45	0.38	0.44	0.18	0.1- 0.8
Prorubricytes	1.47	1.56	1.49	0.48	0.9- 2.6
Basophil rubricytes	4.23	3.00	3.98	1.11	2.0- 6.4
Polychromatophil	13.39	13.74	13.45	3.14	8.5-20.4
Metarubricytes	19.18	22.06	19.76	4.93	13.1-30.4
Total erythrocytic cells	38.72	40.74	39.12	4.67	30.9-48.0
Granulocytic cells					
Myeloblasts	0.61	0.52	0.59	0.22	0.3- 1.1
Progranulocytes	2.51	1.94	2.39	0.90	1.2- 4.5
Neutrophil myelocytes	5.35	5.60	5.40	1.01	3.8- 7.6
Metamyelocytes	4.15	3.68	4.06	0.79	2.6- 5.6
Bands	10.94	10.74	10.90	1.56	8.2-14.6
Segmented	25.24	24.78	25.14	3.16	20.9-30.5
Total neutrophils	45.68	44.80	45.50	4.15	37.7-54.7
Eosinophil myelocytes	0.69	0.94	0.74	0.77	0- 3.4
Metamyelocytes	0.54	0.94	0.62	0.65	0- 3.2
Bands	1.28	1.74	1.37	0.51	0.6- 2.7
Segmented	1.48	1.36	1.46	0.75	0.6- 3.5
Total eosinophils	3.99	4.98	4.19	2.04	2.2-11.7
Basophil metamyelocytes	0.21	0.18	0.20	–	0- 0.6
Bands	0.09	0.10	0.10	–	0- 0.4
Segmented	0.08	0.02	0.07	–	0- 0.3
Total basophils	0.38	0.30	0.37	–	0- 0.9
Total granulocytic cells	53.17	52.54	53.04	4.13	46.3-61.3
Myeloid: erythroid ratio	1.37	1.29	1.36	0.26	0.97-1.86
Other cells					
Lymphocytes	4.56	4.20	4.49	1.18	1.4- 6.7
Monocytes	0.62	0.70	0.64	0.18	0.3- 0.9
Plasma cells	2.93	1.82	2.71	1.21	0.6- 6.1
Mitotic erythrocytic	0.83	0.74	0.81	0.37	0.2- 1.9
Mitotic granulocytic	0.25	0.36	0.27	0.16	0- 0.7

Although hematological values for newborn *M. mulatta* have been reported in the literature, these have been based on very few animals and do not show the progressive changes that occur with maturation. Blood values from birth through approximately 1000 days of age for *M. mulatta* are presented in Table XII.

The study initially involved 29 uninoculated control animals born at the laboratory and hand raised in isolators. The difference between

Table XII

Hematological Values for Uninoculated Infant *M. mulatta*

	19 Animals, 1-2 days old	10 Animals, 3-7 days old	14 Animals, 8-14 days old	18 Animals, 15-21 days old	12 Animals, 22-28 days old	20 Animals, 61-90 days old	20 Animals, 151-180 days old	13 Animals, 350-399 days old	23 Animals, 600-799 days old	25 Animals, 800-1004 days old
Hematocrit (%)	56	55	47	44	43	44	41.5	42	42.5	40
	± 1.4	± 1.7	± 1.7	± 0.9	± 1.2	± 0.8	± 0.9	± 0.9	± 0.5	± 0.5
Hemoglobin (gm %)	17.5	17.2	15.5	14.2	13.8	13.7	12.9	13.0	13.7	13.3
	± 0.35	± 0.49	± 0.65	± 0.19	± 0.36	± 0.23	± 0.53	± 0.38	± 0.20	± 0.17
Platelet (per mm^3)	352	386	617	493	585	442	547	438	382	–
	± 42.8	± 67.8	± 66.1	± 89.0	± 100.2	± 52.5	± 43.3	± 39.5	± 25.5	–
Total white blood cells (per mm^3)	14,710	7,955	10,616	11,778	9,875	9,629	8,843	10,463	10,571	8,526
	± 1,028	± 750	± 1,081	± 790	± 988	± 841	± 867	± 803	± 642	± 463
Differential bands										
Per mm^3	48	15	0	15	4	13	–	68	3	0
	± 18.9	± 15.1	–	± 10.6	± 4.3	± 9.1	–	± 46.6	± 3.2	–
Percent	0.3	0.2	0	0.2	0.1	0.1	0	2.4	0.05	0
	± 0.14	± 0.21	–	± 0.10	± 0.09	± 0.07	–	± 1.65	± 0.05	–
Segmented neutrophils	10,140	4,010	5,212	4,362	3,558	2,523	2,475	3,006	2,300	1,891
Per mm^3	± 908.0	± 555.1	± 751.5	± 455.0	± 719.0	± 481.0	± 345.0	± 653.0	± 294.0	± 267.0
Percent	68.5	49.6	50.6	38.3	35.8	27.4	26.4	28.4	23.5	24.6
	± 3.26	± 4.23	± 4.49	± 2.72	± 4.16	± 2.99	± 2.25	± 6.15	± 3.02	± 3.29
Lymphocytes	3,741	3,257	4,626	6,000	5,221	6,570	5,904	5,585	7,450	6,022
Per mm^3	± 569.0	± 406.0	± 623.5	± 652.5	± 645.0	± 631.0	± 631.0	± 1,094.0	± 585.0	± 475.0
Percent	26.1	41.1	40.6	49.6	52.2	66.5	68.7	54.5	69.1	68.3
	± 3.72	± 4.67	± 4.39	± 2.49	± 4.96	± 3.09	± 2.42	± 7.83	± 2.73	± 3.26
Monocytes	711	476	542	662	555	313	255	344	341	346
Per mm^3	± 127.4	± 92.6	± 118.7	± 113.7	± 113.9	± 65.6	± 62.5	± 75.6	± 70.1	± 38.9
Percent	4.6	6.7	6.1	5.6	6.0	3.6	2.5	3.8	3.2	4.3
	± 0.73	± 1.23	± 1.44	± 0.96	± 1.03	± 0.66	± 0.41	± 0.85	± 0.52	± 0.37
Eosinophils	53	48	178	575	489	203	187	247	438	228
Per mm^3	± 24.6	± 20.7	± 31.7	± 86.5	± 112.4	± 51.8	± 68.9	± 73.1	± 91.0	± 40.2
Percent	0.3	0.8	1.9	5.4	5.5	2.1	2.3	2.4	3.9	2.4
	± 0.15	± 0.29	± 0.45	± 0.98	± 1.07	± 0.64	± 0.57	± 0.84	± 0.64	± 0.38
Basophils	17	7	56	80	44	5	21	59	38	39
Per mm^3	± 11.8	± 6.8	± 20.2	± 31.2	± 20.4	± 3.9	± 16.2	± 21.2	± 12.8	± 12.8
Percent	0.2	0.1	0.7	0.6	0.4	0.3	0.2	0.5	0.3	0.4
	± 0.12	± 0.51	± 0.78	± 0.49	± 0.19	± 0.17	± 0.13	± 0.25	± 0.10	± 0.15

this initial number and the number indicated in the Table reflects the number of animals withdrawn from the study because of clinical illness or death. Counts from animals exhibiting overt clinical signs of pneumonia or diarrhea were also excluded from the study. Babies were bled once weekly for the first 4 weeks and then monthly thereafter.

At the time of birth the hematocrit is at its highest level and gradually drops as the animal ages. It reaches adult levels at about 6 months of age. The average MCV at time of birth is about $100/\mu^3$ and drops to $70/\mu^3$ by 90 days of age. Neutrophils are highest at birth and decline during the first 3 years of life. Lymphocytes, on the other hand, are lowest at birth and increase rapidly to adult values in the first 3 weeks of life. There is a complete reversal of the lymphocyte-neutrophil ratio in the first 4 weeks of life.

The blood counts of babies are characterized by even greater variation than those observed in adults. It will be noted that the standard error in the infant group is two to three times larger than for the adult group. For this reason, it is difficult to evaluate a single blood count and one is limited more to group analysis in the detection of variance.

ADULT MEDICINE

DISEASES OF THE ALIMENTARY SYSTEM

Dysenteric syndromes are a major cause of death in *M. mulatta* being conditioned. Anatomically, the picture is a varied one of a hemorrhagic enteritis, enterocolitis, or only colitis. The intestines are usually empty apart from thick, mucoid, and bile-stained slime; the walls are thickened and edematous. Ulcerating forms are seen. Attempts to isolate specific pathogenic bacteria may fail. Otherwise, probably the commonest causes are *Shigella flexneri* and *Salmonella* sp. enteropathogenic *Escherichia coli* and occasionally *Proteus* sp., *Pseudomonas* sp. and *Aerobacter aerogenes* have been isolated from some cases. In many instances the causes may be multiple because various helminths, amebas, or other protozoa may complicate the picture. Nutritional factors may play a role. Some cases are complicated with lipid metamorphosis of the liver.

Twistleton-Wykeham-Fiennes (1967) deals at length with the various organisms involved. Schneider *et al.* (1960) made an extensive survey of enteric bacteriological findings covering thousands of *M. mulatta* and *M. irus*. The problem of a carrier state is important—probably more important in the case of *Salmonella* than *Shigella*.

As in children, some dysenteric states in monkeys may be succeeded by neurological complications in which there is an ascending paralysis starting in the lower limbs and proceeding rapidly to coma and death (van Bogaert and Innes, 1962).

Shigellosis is characterized by a bloody, often mucoid diarrhea, anorexia, depression, and dehydration with associated electrolyte imbalance. If untreated, the result is coma and death, often within 24 hours. The signs of other bacterial enteritides (*E. coli, Salmonella* sp.) are the same as above except the diarrhea is usually not bloody.

Therapies for bacterial enteritis and nonspecific diarrhea are as follows. Dosages should be adjusted according to the severity of the illness.

The choice of antibiotics is based on bacterial culture and sensitivity. Some commonly used antibiotics and dosages are:

(1) Kantrex (kanamycin sulfate): 7.5 mg/kg intramuscularly twice daily.

(2) NegGram (nalidixic acid): 25 mg/kg twice daily

(3) Chloromycetin palmitate (chloramphenicol palmitate): oral, 25 mg/kg twice daily; 40 mg/kg once daily.

(4) Chloromycetin (chloramphenicol sodium succinate): preferably 15-20 mg/kg intramuscularly three times daily or 25 mg/kg intramuscularly or intravenously twice daily.

(5) Furoxone (furazolidone): preferably 3 mg/kg twice daily or 4-6 mg/kg orally once daily.

Internal protectives and adsorbants are used often with oral antibiotics. Dosage of Kaopectate (kaolin and pectin) varies according to the severity of diarrhea. General dosage is 1-2 ml/kg, twice daily initially and decreased as the stools become firm.

Antispasmodics are used to alleviate gastrointestinal hypermotility. The dosage is extremely variable but generally is 2-6 ml of a 4% opium tincture (paregoric USP).

Perhaps more critical, therapeutically, than the gastrointestinal problems is the sequela of dehydration. Before fluid therapy is used to correct fluid and electrolyte imbalance, the condition must be diagnosed. Signs of marked water and electrolyte deficits are lethargy,

weight loss, "sunken eyeballs," anorexia, wrinkled tongue, loss in elasticity of skin and, in severely affected animals, rapid heart rate and weakened pulse. In treating diarrhea, prompt replacement of lost fluid is imperative (Pickering and Kao, 1961). The use of normal saline alone is inadequate because it is lacking in other ions usually lost during the period of diarrhea (calcium, magnesium, phosphorus, and potassium).

Depending upon the severity of diarrhea, one of the following products can be use to cope with the state of dehydration and electrolyte imbalance.

(1) Ambex is an amino acid solution with electrolytes, vitamin B complex, and dextrose in a dosage of 10-20 ml/kg which is given by any parenteral route as often as indicated.

(2) Dextrose, 2½%, in half-strength lactated Ringer's solution or dextrose with water (5%) is given, 15-25 ml/kg, as often as needed.

A prolapsed rectum is often associated with protracted diarrhea or severe hypermotility of the alimentary tract. This is easily reduced manually and surgically with a purse-string nonabsorbable suture, Vetafil, placed at the anal mucocutaneous junction.

Constipation may result if the monkey is overdosed with Kaopectate or from antispasmodic therapy. For treatment of this condition we employ milk of magnesia or another mild laxative. Enemas are rarely necessary.

Gastritis is generally related to dietary upset and is rarely of bacterial origin. Vomiting is a frequent sign and when the causative factor is removed there is often immediate remission.

Parasitic infestation of the gastrointestinal tract in adult *M. mulatta* is usually more chronic than acute. Thiabendazole has been considered by many investigators to be the drug of choice for elimination of intestinal nematodes.

A study to determine the efficacy of thiabendazole was carried out on 42 adult female monkeys. Three pretreatment and two post-treatment stool specimens from each were examined. Thiabendazole was given at a dose of 100 mg/kg. This dose was repeated 2 weeks later. Post-treatment stool specimens were obtained at least 1 month after the second treatment. The results of this study are shown in Table XIII.

Results of the study tend to indicate that while thiabendazole at this dose is effective in reducing infestation, it does not completely eliminate it.

Table XIII
Effect of Thiabendazole on Reducing Parasitic Infestation in Adult *M. mulatta*

Parasite species	Pretreatment		Post-treatment	
	No. animals positive	Percent	No. animals positive	Percent
Strongyloides sp.	31	74	2	5
Trichostrongylids	29	69	1	2
Oesophagostomum sp.	15	36	4	10
Spiruroids	6	14	0	0
Trichuris trichiura	4	10	1	2
Ternidens sp.	1	2	0	0
Bertiella studeri[a]	1	2	6	14
Anatrichosoma sp.[a]	4	10	10	24

[a]These parasites were not considered in evaluating the effectiveness of thiabendazole. *Bertiella studeri* is a cestode. *Anatrichosoma* is a parasite of the nasal passages; eggs are swallowed and passed through the gastrointestinal tract. There is no conclusive explanation for the increased incidence of these two parasites after treatment.

DISEASES OF THE RESPIRATORY SYSTEM

Pneumonia: In our colony, pneumonia has been the commonest cause of death in newly imported *M. mulatta*. In conditioned animals, and in those established in our breeding colony for years, pulmonary infections have gradually receded as problems largely as a result of improved methods of maintenance, clinical care, and surveillance. Studies of simian pneumonias include Ruch (1959) and Twistleton-Wykeham-Fiennes (1967). In brief, there are two main pathological types, viz., bronchopneumonia and a lobar variety sometimes fibrinous or purulent with abscess formation. It is impossible on an anatomical basis to differentiate various etiological types, and cultural work is a necessity. The most common isolates in our experience have been *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Diplococcus pneumoniae, Streptococcus* sp. Occasionally *Proteus* sp., *Psuedomonas* sp., and other organisms have been isolated. Pneumonia is generally characterized by a reddened face, nasal discharge, dyspnea, occasional coughing, anorexia, abdominal breathing, and wheezing with depression in advanced cases. Occasionally, a foreign-body pneumonia results from inadvertent drug intubation into the trachea and bronchi.

Therapy, if instituted early, is very rewarding, and generally consists of the following antibiotics:

(1) Keflin (cephalothin): 20-40 mg/kg intramuscularly twice daily.

(2) Lincocin (lincomycin HCl): 5-10 mg/kg intramuscularly twice daily.

Various penicillins and Chloromycetin are also used and, occasionally, sulfonamide drugs such as Bactrovet (sulfadimethoxine). Other supportive therapy depends upon the severity of signs. In cases of severe dyspnea, atropine for bronchial dilation is given (0.2–0.4 mg total dose) with oxygen and fluid therapy.

A variety of nematodes and trematodes infect the lungs of apes and monkeys; hydatid disease has also been recorded (for simian species susceptible, see Ruch, 1959). Several viruses have been recovered from simian lungs, but their pathogenicity is not well understood. An interstitial pneumonitis, associated with giant-cell formation and intranuclear and cytoplasmic inclusion bodies, occurs in young animals and adults. Some cases are certainly associated with the measles virus.

Tuberculosis: Whenever colonies of monkeys are maintained for research, tuberculosis still constitutes a major disease problem and if monkeys are housed in batches it can ravage a colony. Habel (1947) reported that about one-third of all monkeys received at the National Institutes of Health, Bethesda, Maryland, developed tuberculosis and he produced unequivocal evidence on the rapidity with which the disease spread. In 290 monkeys the mortality was 58%. No reactor to the tuberculin test lived longer than 38 weeks and once infection was established in the colony there were 18% reactors to the tuberculin test at 3 months, 57% at 6 months, and 100% at 9 months.

Habermann and Williams (1957) at the same institution, found the situation was still serious; they found 101 cases of tuberculosis in necropsies of 615 *M. mulatta*, but none in 93 cynomolgus monkeys. Benson, Fremming, and Young (1955) described an outbreak in another laboratory necessitating disposal of the entire colony of over 200 monkeys. Schroeder (1938a) estimated that the average death rate from tuberculosis in imported monkeys in zoological gardens and laboratories was about 10%, and that about 80% of all deaths were attributable to this disease. Schroeder (1938b) also introduced the tuberculin test as a diagnostic aid in simians.

Other important contributions to the literature on simian tuberculosis are by Francis (1958), Ruch (1959), van Bogaert and Innes

(1962), Innes (1963), and Twistleton-Wykeham-Fiennes (1967). Some other informative original studies, extensively quoted in the above sources, were those by Scott and Beattie (1928), Scott (1930), Nieberle (1932), Schmidt *et al.* (1955), and Schmidt (1956).

There are variations in susceptibility between different kinds of simians, e.g., between *M. mulatta* and *M. irus*, but the former has the greatest incidence of reported cases which may be partly because of the fact that it is the commonest species used experimentally. Francis (1958) stated that in 123 records of typed cultures of *Mycobacterium tuberculosis* recovered from simian cases, 89 were of the human variety, 28 bovine, and 6 avian. The latter variety can be of little practical significance and bovine tuberculosis now has been largely controlled in the United States and most of Europe. Further, the onetime common practice of giving raw milk to monkeys has ceased. Twistleton-Wykeham-Fiennes (1967) commented on the fact that in the London Zoological Gardens there has been no case of tuberculosis resulting from the human type for 6 years, but an occasional case attributable to the bovine bacillus has occurred. Little has been written about the latter except that it causes mainly alimentary infection with involvement of the regional lymph nodes. Reference should be made to the studies of Schmidt *et al.* (1957) on the pathogenicity of atypical chromogenic mycobacteria for *M. mulatta*. The general opinion is that monkeys (*M. mulatta*) do not acquire infection in their natural wild habitat but only when they first come in contact with human sources of infection and thereafter spread the disease to other monkeys.

In our experience, most cases have occurred in monkeys in the Conditioning Colony, but very rarely in established breeders and never in monkeys born in our laboratories. We do not know what the incubation period is in tuberculosis of monkeys. One serious problem which has confronted us is cases occurring in a room of monkeys that had been repeatedly tuberculin-tested and in which no identified source of contagion was known. The validity of previous negative tuberculin tests must then be questioned.

The reliability of the test has been questioned time and again. We know that in the very early stages and in the terminal phase the test may be negative. Further, it is recognized that in monkeys (as in other animals and in man) the presence of nonpathogenic mycobacteria in the monkey body will produce a positive reaction and a similar sensitivity follows vaccination with BCG (Schmidt, 1967). The tuberculin test in man and animals is still a fertile field for investigation. The use

of avian and bovine PPD tuberculins in comparative tests in cattle have proved of value (Worthington and Kleeberg, 1960). Trolldenier *et al.* (1961) showed that in monkeys the tuberculin sensitivity of the skin of the forearm was a better site than the eyelid for injection with 100 and 1000 units of tuberculin.

Within a closed colony, infection is not necessarily acquired by repeated body contact with other animals. This was proved by Innes (1963) who reported three cases in monkeys that had been kept in separate cages under excellent care for 26, 33, and 24 months, respectively, after receipt from a dealer. The conclusion was that infection must have been airborne from an unidentifiable source.

The tuberculin test is dealt with below. Obviously, all reactors should be killed and a necropsy performed to confirm the diagnosis by examination of smears and sections stained by the Ziehl-Neelsen method for acid-fast bacilli. Because of expense, we do not now attempt to isolate mycobacteria and type cultures, for there is little point to that unless a special study of simian tuberculosis is being made. We do little more now than to search for a primary lesion, first in the lungs and then in the regional pulmonary lymph nodes (hilar and bifurcation). Occasionally, no macroscopic primary lesion will be found in the lungs, but only in the regional nodes. Retropharyngeal areas, tonsils, intestines, and regional nodes should be examined closely if no primary pulmonary lesions are found in the usual necropsy routine. Sections are made of all lesions.

Once primary infection is established and before early generalization, the lesion is a single caseous focus, varying in size up to a centimeter or more and usually situated in a subpleural area. The further progress of the disease indicates that what we see in the simian disease is a close analogy to primary tuberculosis of children with its frequent and early forms of postprimary generalization, or what was once seen in adult human populations in certain parts of the world after they came in contact with infection for the first time. Chronic isolated tuberculosis (phthisis) of the human adult, resulting from reinfection, has not been seen by us in monkeys but reference must be made to the studies of Schmidt and his colleagues from 1955 onward.

The disease process starts to spread within the lungs partly by aspiration to other parts via the bronchiolar pathways, and via the intrapulmonary circulation. By lymphatic drainage paths, the regional lymph nodes become involved. Thereafter, by bacillary invasion of the

bloodstream, via arteries and veins, bacteria can be transported to many different organs throughout the body, and this process may be repeated many times until the death of the animal. The process may result in large and small nodular lesions in the liver, spleen, and other organs or in the classic form of miliary tuberculosis in which a large number of organs are literally seeded by minute lesions. The nervous system is probably involved far more frequently than the literature suggests (see van Bogaert and Innes, 1962; Innes, 1963) if trouble is taken to examine the brain and spinal cord.

In simian lesions there is progressive central caseation, rarely calcification, with tissue reaction in which epithelioid cells predominate and Langhans' giant cells can be either absent, rare, or very numerous. By search in Ziehl-Neelsen-stained sections, acid-fast mycobacteria can be identified in variable numbers; sometimes they are scanty and sometimes, extremely numerous. In monkeys, not all such epithelioid and giant cell lesions with central necrosis are tuberculous. Some may be those of nocardiosis (Jonas and Wyand, 1966). The possible confusion of tuberculous lesions with those associated with lung mites was discussed by Innes *et al.* (1954) but histologically there can be no mistake.

Clinically, the signs are not conspicuous until the disease is very advanced, and it is surprising how often widespread disease is found involving many organs in an animal that does not appear very sick. (Ruch gives a good account of some clinical findings.) However, for diagnosis when tuberculosis is suspected, reliance must be placed on use of the tuberculin test and radiographs of the chest.

All our monkeys are fed isoniazid (INH or isonicotinic acid hydrazide) daily on a sugar cube as a prophylactic measure against tuberculosis at a dose of 2.5 mg/kg B.I.D. (see Schmidt, 1956, 1959). The isoniazid solution is prepared by dissolving 9.1 gm of isoniazid powder in 100 ml of water. One drop of solution equals approximately 6 mg and an appropriate number of drops are absorbed onto sugar cubes.

In the tuberculin test of monkeys we use tuberculin (old tuberculin) in a 1:9 dilution in sterile water. One-tenth ml (10.0 mg of tuberculin) is injected intradermally into an upper eyelid and observations are made at 24, 48, and 72 hours. A positive reaction is evidenced by erythema and massive edema of the eyelid which often close the eye with matted secretions (Fig. 22).

An anergic case is rarely seen, perhaps 1 in 500 *M. mulatta*. When seen, it is generally among a recently received shipment (because of

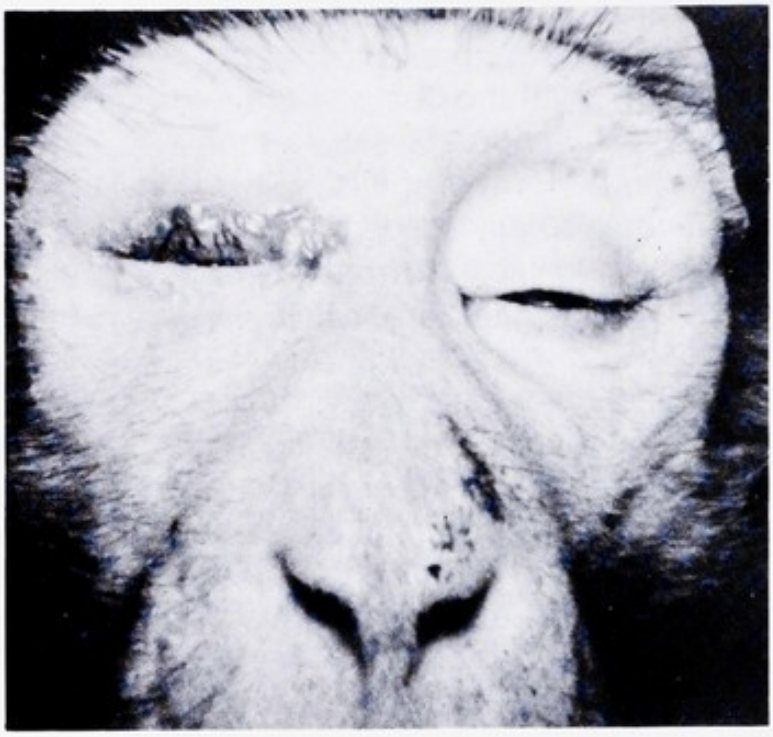

Fig. 22. Tuberculin test, positive reaction after 72 hours. Note the edema and encrustations on upper right eyelid.

the acute course of the disease). Although these monkeys betray no sign of reaction when tested, they rapidly become moribund and tuberculosis is detected at necropsy. Recently, we have begun to employ x-ray examination of the chest when clinical signs suggest any suspicion of pulmonary tuberculosis. We have never carried out systematic work with the use of BCG as a vaccine in monkeys; extensive experimental studies have been carried out by Schmidt (1967).

Pulmonary acariasis: This disease of the lungs of *M. mulatta* cannot be dismissed as of no account, although it is not a clinical entity (i.e., not diagnosable). However, its occurrence in almost every member of this species imported from India indicates that it is a problem – the extent to which it affects body health is impossible to ascertain. It should be emphasized that in simian progeny reared in our colony the infection has never been seen, meaning that removal of the baby from the mother destroys the link of infection. The disease is covered by Innes *et al.* (1954).

The implicated mite is *Pneumonyssus simicola*, other species of which infect other animals (and man). Clinically, the disease may not cause any tangible signs, nor are radiographs of the chest of much

value for diagnosis. The mites, by their wanderings in the lungs, must irritate the bronchial passages and interfere with normal health, reducing pulmonary capacity.

Macroscopically, the lesions are unmistakable and may be found in any or all lobes and range in number from a very few to over 100. They vary in size from a pinhead to a few millimeters or more in diameter and somewhat simulate focal mycobacterial lesions. Sometimes they become confluent; rarely do they produce lobar pneumonia but many have "violin-string" fibrous adhesions to the pleura. Under the dissection microscope they are jellylike masses with a small slit in the center from which mites can be removed. Adjacent lung tissue shows a marbling effect resulting from black pigment, which is not anthracotic but is hemosiderin and other products of the mite excreting digested blood. The lesions are essentially bronchiolitis and peribronchiolitis, sometimes severe, with a reaction mainly of lymphocytes and macrophages containing pigment and crystals; eosinophils may predominate. Mites may be found in sections of a lesion.

Nothing is known about the pathogenesis or how monkeys acquire infection, but the most likely explanation is that the parasites are picked up and passed via the nasal cavities (where they have been found) to the lungs.

All life stages of the parasites have been found in lungs, adult males and females, eggs, and larvae. Cases with multiple disseminated lesions could represent repeated exogenous infections from nose and mouth contact with other affected animals but they could also be repeated endogeneous reinfections by fresh hatchings, larval maturations, and new migrations.

No methods of control are known other than to rear babies in isolation from their mothers.

DISEASES OF THE CARDIOVASCULAR SYSTEM

In monkeys, systemic hypertension is the most frequent cardiac disease and arteriosclerosis is the least common. Myocardial infarction is rare; acute and chronic endocarditis have been reported without identification of cause. Mitral stenosis with regurgitation and diffusely contracted, fused mitral leaflets, and chordae tendinae in two monkeys was described by Lapin and Yakovleva (1963) as a probable infective endocarditis which had healed. Atherosclerosis was discussed by Malinowa (1965) who found the disease in a fair number of

M. mulatta. One of the animals was a hypertensive male, 27 years old.

Two cardiac cases were found in our colony in 5 years (Roberts and Innes, 1966). In the first case the animal had a scarred mitral valve, which was both stenotic and incompetent; in the second case, the animal had pancarditis with acute endocarditis of both mitral and aortic valves leading to aortic stenosis. The etiology was not determined. Neither organisms nor Aschoff bodies were found in histological sections, but neither rheumatic disease of the heart nor Aschoff bodies has ever been reported in any animal other than man. There was no clinical history available for either monkey, but 12 hours before death the first animal was in a state of severe congestive cardiac failure with ascites and tachycardia (220 beats per minute).*

DISEASES OF THE NERVOUS SYSTEM

No specific disease† of the nervous system that could be indisputably linked with some basic neuropathological disorder has been found by us in our colony in some 5 years of experience with a very large number of *M. mulatta*, both adults and infants. In spite of their close phylogenetic relationship, apes and monkeys do not suffer from the legion of neurological disorders that afflict *Homo sapiens*. However, as van Bogaert and Innes (1962) showed, they can be affected with a restricted but remarkable variety of neurological diseases, some of which are species-specific (e.g., leucoencephalomyelosis and acute amaurosis with and without epilepsy).

Further, for a long time, simians have been used for extensive experimental work on many viral encephalitides affecting other species, notably poliomyelitis – see van Rooyen and Rhodes (1966), Bodian (1948), and Hurst (1929). Consequently, in a chapter on simian medicine, it is at least advisable to give some indications of the literature available.

For all aspiring simian clinicians, the essay by van Bogaert (1962) on clinical neurological observations on apes and monkeys is essential study. Data on simian cerebrospinal fluid are given in van Bogaert and Innes (1962).

Consideration of simian neurology and neuropathology requires

*Another case was observed in our colony (Ulland, 1968). Our eighth oldest baby, about 5 years old, died with cyanosis and a fainting attack. At necropsy there was thrombosis of the pulmonary trunk and right artery with consequent pulmonary infarction.

†Only one concomitant (and not specific) disease has been observed which was a fatal, very acute, suppurative, part hemorrhagic meningoencephalitis caused by *E. coli*.

prior appreciation of neuroanatomy. For beginners there are good plates in van Bogaert and Innes (1962). More detailed books and atlases include: Krieg (frontal connections, 1954); Riley (cerebellum, 1929); Dow and Moruzzi (cerebellum, 1958); von Bonin and Bailey (neocortex, 1947); Connolly (external morphology, 1950); Monnier (brain stem, 1949); Hrdlička (brain weights, 1905, 1925); Snider and Lee (1962), *A Sterotaxic Atlas of the Monkey Brain* (*Macaca mulatta*); Ariëns-Kappers *et al.* (1936); and Tilney (1928). An atlas of electroencephalographic recordings on the normal *M. mulatta* of all ages, as well as on some experimental conditions, was produced by Caveness (1962).

A full account of B-virus infection in man and monkeys is given by van Bogaert and Innes (1962) and the best original account of the disease, with its herpetic manifestations in monkeys, is by Keeble *et al.* (1958). The disease is of supreme importance to all who work with monkeys in that it is transmitted to man by bites (or saliva onto cuts and scratches) and is probably universally fatal as an ascending myelitis.

It is important to note that the first case of natural rabies in *M. mulatta* was reported in 1966 (Boulger, 1966). The signs started on the forty-seventh day after importation, but the animal showed no aggression, no aversion to drinking, and no paralysis; it did persist in severe self-mutilation of fingers and hands. Rabies must be added to B virus and other infections that constitute extreme hazards for all those handling newly imported animals.

MISCELLANEOUS DISEASES OF ADULTS

Rashes of the skin and other lesions associated with measles, monkey pox, yaba-like viral infection, and other infectious agents appear from time to time but they occur primarily in newly imported animals. The characteristic skin lesions of monkey pox were described by Ruch (1959). We have seen no proven cases of B-virus herpetic lesions of mouth, lips, or gums. A common affliction in our colony has been dermatological lesions caused by coagulase-positive *S. aureus*, mostly penicillin resistant. Keflin and Lincocin have been therapeutically successful.

Nephritic cases are almost unknown to us but one case of glomerulonephritis attributable to *S. aureus* was diagnosed.

One case of diabetes mellitus has been observed by us in *M. mulatta*. A middle-aged female delivered the largest baby born in our laboratory, a female infant weighing 849 gm. Because in man new-

born infants of overtly diabetic mothers are often overweight and appear Cushingoid, a fasting blood glucose and glucose tolerance were performed with the following results: fasting, 218 mg%; 1 hour, 396 mg%; 2 hours, 387 mg%, 3 hours, 147 mg%.

Normal fasting blood glucoses in *M. mulatta* range between 60 and 110 mg%. Three months postpartum, repeated tests indicated substantial reduction of the glucose level to near normal. We have no precise explanation for this phenomenon.

We have seen no cases of undisputed deficiency disease and certainly no animals with rickets or scurvy.

One case of a severely crippled monkey (adult) was found with widespread osteoarthritic lesions of the joints of the upper and lower limbs and with some degree of spondylitis deformans.

In 6 years only one tumor has been found, viz, a granulosa cell tumor of the ovary, removed surgically without recurrence or metastasis so far after 2 years. However, in our colony the adult monkeys are still not very old (i.e. of the presumed cancer age). References to simian neoplastic diseases are given in Ruch (1959), O'Gara *et al.* (1967) and Valerio *et al.* (1968).

PEDIATRIC MEDICINE

Simian babies and juveniles are afflicted from time to time with common disorders such as gastritis, enteritis, pneumonia, other bacterial and viral infections, anemias, complications resulting from aspiration of material into the lung, as well as other illnesses. A substantial survey of animals born within the colony has revealed, to date, no evidence of helminthic infestation. This chapter describes some of the conditions that frequently face the veterinarian charged with maintaining the health of young simians and suggests appropriate therapies. Treatment outlined here applies to simians up to about 3 years of age.

More of the bacterial pathogens in a simian colony are gram-negative than gram-positive. If an animal suddenly develops diarrhea due to *Pseudomonas aeruginosa*, the drug of choice is polymyxin B (polymyxin B sulfate, 0.75 mg/kg three times daily). It is generally effective against *E. coli*, *S. flexneri*, and others, but since it is often implicated in nephrotoxicity, its use is restricted to *P. aeruginosa* infection. Proteinuria indicates nephrotoxicity resulting from polymyxin B; thus, urine protein is checked twice daily during therapy.

Generally, the best drugs for general therapy against gram-negative organisms are Kantrex, NegGram, Furoxone, and Chloromycetin. NegGram, an orally administered preparation, is effective for many enteric organisms, and is also effective for infectious diseases of the urogenital system. Keflin and Lincocin (lincomycin hydrochloride, 5-10 mg/kg twice daily) are excellent drugs used mainly for *S. aureus* which is frequently penicillin resistant.

Other antibiotics commonly used for gram-positive organisms (such as *Diplococcus pneumoniae* and *Streptococcus*) causing pneumonia and upper respiratory infection are Longicil (benzathine penicillin G and procaine penicillin G, 30,000 units/kg), and many newer antibiotics which include Polycillin-*N* (ampicillin sodium), erythromycin, and Unipen (nafcillin sodium). Liquamycin (Terramycin) is of limited value in treating simians and may cause toxic effects. Dihydrostreptomycin is used when it is the only effective antibiotic for *Proteus* based on sensitivity. Biosol (neomycin sulfate) an oral preparation administered for 2-4 days will cause a sterility of the alimentary tract with resulting diarrhea. Bactrovet (sulfadimethoxine, 20 mg/kg reduced one-half on succeeding days) is a long-acting (24 hours) sulfonamide. It affords excellent protection against a broad spectrum of organisms. Tylocine (tylosin) is another veterinary drug useful against gram-positive organisms in either the intestinal or respiratory tracts.

Mycostatin (nystatin) is probably the best agent for thrush, an oral fungal infection caused by *Candida albicans* and seen frequently in baby simians.

A premature animal is placed on antibiotic therapy for 2-4 days if it is suspected that the baby was born prematurely as a result of maternal infection. This course of action is determined by the clinical picture of the mother at or prior to delivery and is followed as a prophylactic or precautionary measure in the infant.

DISEASES OF THE ALIMENTARY SYSTEM

Gastroenteritis and enteritis are generally caused by various pathogenic bacteria. Some of the more common pathogens encountered by us have been *S. flexneri* and less frequently, other *Shigella*, such as *S. sonnei*, various species of *Salmonella*, enteropathogenic *E. coli* of group B., *P. aeruginosa*, *Proteus*, *Enterococcus*, *Aerobacter*, and *S. aureus*. Gastritis is often caused by dietary problems. Diarrhea, anorexia, vomiting, abdominal pain induced by flatulence and loss of skin elasticity are probably the most frequent signs associated with these enteric problems. Diarrhea is probably the most common sign

of enteritis, as well as the most frequently encountered clinical disorder among young simians. Signs vary from mild to profuse diarrhea. As a result, dehydration, electrolyte imbalance, and their sequelae such as shock may further complicate the situation. Treatment should be based on the severity of the signs as well as etiology determined by bacterial culture and antibiotic sensitivity test.

In the cases of mild diarrhea, the animal should be given Kaopectate (kaolin and pectin, 1-3 ml/kg orally), intravenous or subcutaneous fluids as necessary and antibiotics. In cases of severe diarrhea, Kaopectate (2-5 ml/kg) may be given both orally (flavored with cherry syrup) and intrarectally. Intravenous or subcutaneous fluids should be given three times a day for as many days as warranted. If severe bacterial enteritis is encountered, oral or parenteral antibiotics are given immediately. Antibiotics commonly used, based on culture and sensitivity tests are Chloromycetin (chloramphenicol palmitate, 5 mg/kg, orally four times daily); Chloromycetin (chloramphenicol sodium succinate, 8 mg/kg, intramuscularly or intravenously three times daily); Furoxone (furazolidine, 1.5 mg/kg, orally three times daily); Kantrex (kanamycin sulfate, 5 mg/kg intramuscularly twice daily); NegGram (nalidixic acid, 15 mg/kg, orally).

Atropine (0.05-0.08 mg/kg) and paregoric (2-3 drops) are used to decrease the motility of the gastrointestinal tract. Sustagen, a complete therapeutic nutriment, may be added to, or substituted for, the animal's diet if anorexia and mild dehydration are present. In all cases of diarrhea, food should be eliminated for the first 24 hours of therapy. Fluids, including electrolytes and dextrose, are given parenterally to maintain water and caloric needs.

Emesis: Another problem often accompanying gastritis and gastroenteritis is vomiting. In diagnosing the cause in simians, it is important to consider the animal's diet, gastric distension, and possible stress factors. Many times, simians will eat despite the fact they are bloated and vomiting may result. Vomiting can usually be controlled by phenobarbital (3-6 mg/kg). Thorazine (chlorpromazine hydrochloride, 1-2 mg/kg has recently been used since it has excellent antemetic activity. If emesis is mild, antacids such as milk of magnesia and sodium bicarbonate are employed. Fluid therapy generally used is 0.9% sodium chloride solution or regular Ringer's solution since the primary ion lost is chloride. Pepto-Bismol may be given after the vomiting has stopped. Within 12-36 hours, the animal can be taken off the drugs and restored to a normal diet.

Dehydration: Loss of elasticity of skin and "sunken eye balls" are generally manifest in an animal with moderate to severe dehydration. The latter, and electrolyte loss, often precipitate shock and can be the sequelae of either diarrhea or vomiting. Syncope and dyspnea with resulting cyanosis may accompany shock; in such cases, oxygen, fluid, and electrolyte therapy are employed. Heat is also important for animals recovering from the shock syndrome and is provided by a conventional heating pad placed on the floor of the isolator. Emergency drugs including an analeptic, e.g., Dopram (doxapram hydrochloride, 1-2 mg/kg intravenously or intramuscularly) and a cardiac stimulant, adrenalin (0.2 ml/kg of a 0.001 solution) should always be available. Animals with anorexia and mild dehydration, but without acute diarrhea, may respond to fluid therapy. The preparations often used are Ambex (see p. 92) or half-strength lactated Ringer's solution in 2.5% dextrose. We do not give 5% dextrose solution subcutaneously, although some investigators have used it without ill effect. In very ill infants, poor circulation and other factors cause inadequate absorption from the subcutaneous site. Fluid therapy is dictated by the clinical condition. Some simians apparently tolerate more than the generally accepted limit of their body weight in fluids without developing pulmonary edema. Although some authorities recommend no more than 20 ml of fluid per kilogram of body weight per day, in our experience simians benefit from higher doses. Simians are started at 10 ml/kg twice a day, and the amount and frequency are altered as necessary.

Flatulence: Flatulence may accompany gastroenteritis; it can cause abdominal pain and is best treated with Pepto-Bismol. A sequela of enteritis therapy may be constipation which is treated with milk of magnesia.

DISEASES OF THE RESPIRATORY SYSTEM

Pneumonia presents itself as a serious and relatively common ailment in the newborn *M. mulatta*. The most frequent causes identified have been *K. pneumoniae, Streptococcus, S. aureus, Proteus, Pseudomonas*, and *D. pneumoniae*. Other causes are aspiration of amniotic fluid during delivery and aspiration of food following regurgitation. Nasal discharge, dyspnea, and abdominal breathing are common clinical findings.

In severe respiratory distress immediate treatment, for dilating the

bronchi and bronchioles and suppressing secretions, is the administration of atropine. Antibiotic therapy should be given based on bacterial culture and sensitivity. The antibiotics of choice are Keflin (cephalothin, 15-20 mg/kg, intramuscularly three times daily); polymyxin B (polymyxin B sulfate, 0.75 mg/kg, intramuscularly three times daily); Bactrovet (sulfadimethoxine, 20 mg/kg once daily, thereafter, 10 mg/kg), and a number of the penicillin preparations.

Oxygen is of value if the animal is very dyspneic or cyanotic. For brief intervals the oxygen tube can be placed directly in the animal's mouth and flow regulated to 4-6 liters per minute. If the animal is in an isolette inside an isolator, flow to the isolette can be 6-10 liters per minute. Oxygen flow is gradually reduced as the animal improves.

MISCELLANEOUS DISEASES OF THE YOUNG

Anemias: Often the first indication of anemia in the infant simian is discovered as a result of a routine blood count. The anemia is usually asymptomatic and will generally respond to vitamin, mineral, and dietary supplement.

Occasionally, anemia may result from internal hemorrhaging, in which case Koagamin may be useful (0.1-0.2 ml/kg, intravenously or intramuscularly). Vitamin K may also be used to alleviate hemorrhage.

As prophylaxis against anemia, Similac with iron should be included in the diet at about 2 weeks of age, or when the animal begins to self-feed.

Extremely severe anemia has been encountered as part of a starvation-water deprivation syndrome. Anorexia, depression, dehydration, and a severe hemorrhagic diarrhea may be the initial clinical signs. Water deprivation is the primary cause. Therapy consists of fluid replacement, amino acids (Ambex) multiple vitamins (Poly-B-C) and crude liver-B_{12}-folic acid administration. Antibiotics and antidiarrheal therapy is also indicated since the hemorrhagic diarrhea is frequently associated with an overwhelming *Shigella* infection.

Convulsions: Disorders of this nature are most frequently associated with experimental infants inoculated with viral or bacterial organisms. Cases of encephalitis or encephalomyelitis have occurred with concomitant convulsions which were probably attributable to the experimental inoculum. There have been some convulsions of uncertain etiology and idiopathic epilepsy was suspected. This is yet to be documented.

Convulsions are generally of short duration and occur infrequently. An animal experiencing a convulsion should be given phenobarbital of sufficient dosage to stop the convulsions (4-8 mg/kg, intramuscularly twice daily) and then allowed to rest. If barbiturate toxicity occurs, animals generally respond to Dopram and oxygen therapy.

Some authorities favor giving pentobarbital intravenously to induce the initial quiescence of the convulsions, then maintain sedation with phenobarbital. However, in our experience, intravenous pentobarbital is not necessary as we have found that phenobarbital intramuscularly acts quickly enough to minimize the severity of the convulsion. The phenobarbital dosage should be maintained as long as indicated.

Allergy: Allergies are infrequent among simians, and the causes are generally unknown; however, dietary and drug hypersensitivities have been seen. Allergy usually presents itself as a skin rash. Treatment consists of the administration of an antihistamine such as Chlor-Trimeton (chlorpheniramine maleate, 1-2 mg/kg per injection). Neo-Delta-Cortef ointment or Neo-Decadron cream are applied topically to rash and have been quite effective.

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VII SURGERY

Surgery is a technique used to correct or eliminate signs arising from disease, trauma, or congenital defects. In a research facility, surgical procedures are also frequently used in the conduct of scientific investigations. The surgeon in a simian colony must have an understanding of anatomy, physiology, bio-chemistry, clinical medicine, and pathology, upon which is superimposed an accumulation of skills built by experience. However, surgery is also an art and only its fundamentals can be taught in a formal fashion. It is surgical skill that makes the difference between a successful and unsuccessful operation, and these skills in simian surgery are only acquired through individual effort and practice.

Our major surgery includes: cesarean section, herniorrhaphy, open and closed reduction of fractures, eye enucleation, amputation of extremities, gastrotomy, resuture of disrupted wounds, nephrectomy, thymectomy, splenectomy, laparotomy, hysterotomy, and orchidectomy. Minor surgery includes operations for suture of laceration, amputation of digits or tail, rectal prolapse, vaginal prolapse, drainage of abscess, debridement of wound, dilatation and curettage, canine tooth extraction, excision of tumor, biopsy, tracheotomy, and amniocentesis.

PREOPERATIVE AND POSTOPERATIVE CARE

For all except emergencies, animals are examined for the surgical condition indicated and to determine that overall health is sufficient for surgery. A hematological study is made before surgery; an abnor-

mal red or white cell count may contraindicate surgery. An acceptable preoperative hematological picture for *M. mulatta* is given on p. 85.

If a general anesthetic is employed, the animal must not receive food or water for at least 12 hours before surgery.

The animal is weighed, taken to the preparation room, and restrained on an operating board. It is given atropine (1/150 grain or 0.4 mg total, intramuscularly) one-half hour before surgery, with other medication dependent upon the condition of the animal and the surgical procedure involved.

The surgical site is closely clipped or shaved and scrubbed with a bactericidal detergent using gauze. The animal is transferred to the major surgical room where the operative field is washed with alcohol and ether and sprayed with tincture of Zephiran. The skin site is draped with a sterile sheet fastened to the skin with towel clips. Packaged paper sterile drapes with an opening for the operative site are used. Whenever practical, anesthesia is begun during the surgical preparation.

In special operations, the preparatory procedure may be altered. For example, for the correction of rectal or vaginal prolapse, or for dilatation and curettage, the antiseptic spray is omitted. Special techniques must be adopted for such procedures as canine teeth extraction.

The animal may be anesthetized before, or during, any stage of the preparation. In a situation in which canine teeth must be extracted from a large, aggressive male simian, it is best to anesthetize before the surgical preparation. In cesarean section, it is better to delay general anesthesia as long as possible to avoid depression of the infant.

Prior to gas anesthesia—and sometimes during—ophthalmic ointment or drops shoud be put into the eyes to alleviate possible irritation of the cornea. During all surgical procedures that involve general anesthesia, intravenous fluids are administered.

Following surgery, the anesthetized animal is placed in a cage in the recovery room, on its side to minimize difficulty in breathing. It must be observed frequently until consciousness is regained. A regular diet may be resumed the following day in most cases, except after gastrointestinal tract surgery. In such cases, liquids and soft foods are given. Following teeth extraction, soft foods are given for a week, or longer if necessary.

ANESTHESIA

LOCAL

A local anesthestic is satisfactory for many short minor operative procedures and involves hypodermic infiltration of the operative area with 2% procaine solution.

GENERAL

General anesthetic can be administered by inhalation or by intravenous injection. The intramuscular route should only be used for tranquilizers and neuroleptanalgesic drugs.

In inhalation anesthesia, a volatile anesthetic, such as Metofane (methoxyflurane) is given via an Ethaire anesthesia resuscitation unit. After induction is produced, usually in 3-5 minutes, the trachea is intubated with the aid of a laryngoscope. The endotracheal tube has a double lumen: one for breathing and the other leading to a bulb which when inflated will block off the trachea to prevent fluid, vomitus, or foreign bodies from passing into the lungs. The breathing lumen is attached to the small, anesthetic-mixing apparatus, which can be adjusted to deliver a controlled mixture of anesthetic and oxygen, or room air, without the animal rebreathing the mixture. Intubation assures a patent airway and permits resuscitative measures. The technique usually requires the presence of more than one person because, although under anesthesia, the animals often struggle. The esophagus may also be intubated with a similar tube. Inflating the bulb of the tube blocks off the esophagus to prevent regurgitation of stomach contents.

Inhalation anesthesia is satisfactory for operations of both long and short duration and permits good anesthetic control and prompt recovery. It may be combined with either local anesthesia or with a short-acting intravenous drug such as Pentothal Sodium.

Intravenous anesthesia consists of injecting a short- or longer-acting anesthetic drug into the saphenous vein, and we use the short-acting drug, Pentothal Sodium. The dose—controlled by the reaction of the animal—is discontinued when anesthesia is produced. If used first in conjunction with inhalation anesthesia, the struggling of the animal during intubation is eliminated.

The longer-acting intravenous anesthetic used is Nembutal (pentobarbital sodium) at a dosage of 25 mg per kilogram of weight, injected slowly until anesthesia is produced. Sometimes it is not necessary to inject the full dose. The drug is easily administered, produces deep anesthesia, and is satisfactory for longer operative procedures. The recovery time is 4-8 hours, during which the animal should be observed frequently for obstructed breathing and to avoid aspiration pneumonia or other complications. To assure a patent airway, intubation should be used with all such long-acting anesthetics.

The intramuscular route is used for injection of neuroleptanalgesic and tranquilizing drugs. Innovar-Vet (Sublimaze and Inapsine) has been used by us in a large number of abdominal surgery cases. It obtains full effect in 8-15 minutes. Analgesia lasts 30-60 minutes and tranquilization lasts several hours. The animal remains fully conscious and yet is quiescent, immobile, easy to handle, and has reduced response to painful, tactile, and auditory stimuli. The dosage of Innovar-Vet is 0.1 ml per kilogram.

During any type of anesthesia, the vital signs—pulse or heart rate, respiration, blood pressure, color—should be monitored. Expensive machines or equipment for this purpose, of course, have their advantages, but are not essential. The surgical team should be ready for emergencies and have resuscitation equipment and supplies at hand, such as oxygen, respiratory and cardiac stimulants, and specific analeptics.

OPERATION PROCEDURES

Persons serving as members of the operating room team should be carefully instructed in preparation before surgery. Proper aseptic preparation of the surgical team and proper sterilization and handling of all instruments and supplies used at the operating table cannot be overemphasized. The wearing of a uniform and cap and the masking of nose and mouth are important and minimize the chance of infection.

The operator should remember that in simians, as in humans, general factors of nutrition and age influence the healing of tissues and wounds. Emaciation, malnutrition, hypoproteinemia, anemia, prolonged infection, changes in body chemistry, and vitamin deficiency delay healing. Complications such as coughing, sneezing, hiccoughing, and restlessness also delay healing and may produce wound dis-

ruption leading to infection and tissue necrosis. Correction and proper control of these general factors will contribute greatly to successful surgery and minimize surgical complications and mortality.

A number of factors involving surgical techniques also influence tissue healing and thereby contribute to or detract from the surgical results: proper choice of incision; avoidance of infection and operative trauma; adequate hemostasis; careful choice of suture material; elimination of tissue dead space and foreign bodies; preservation of adequate blood supply; avoidance of wound tension; and the accurate approximation of tissues and wound edges. Many factors influence the quality of surgery.

CESAREAN SECTION

Cesarean section is one of the most frequent major operative procedures performed on simians by us. In order to minimize depression of the infant, local anesthesia can be given with general anesthesia administered immediately after removal of the fetus from the open uterus. If administered before delivery of the pregnant uterus, intravenous anesthetics are less suitable for the procedure because of the infant depression caused by barbiturates. Gaseous anesthetics have been found to produce less fetal depression.

A low midline abdominal incision is usually used. As a general rule, the pregnant uterus is not delivered through the incision. The site for needle puncture for amniocentesis should be determined by palpation of the uterus to avoid trauma from the needle to the infant or placenta. The uterus will often be found slightly rotated and attempts to correct this are not necessary. The uterine incision is transverse and should be placed just inferior to the uterine attachment of the round ligaments. The uterine incision is gently spread with the fingers and the infant quickly delivered. The infant is stimulated to breathe by a few slaps on the back while being held by the feet in an inverted position to drain mucus or fluid from the trachea (Fig. 23).

Meanwhile, removal of the bidiscoidal placenta and membranes—accomplished carefully and completely—can be facilitated by delivering the uterus through the abdominal incision and everting it. Wiping away, or peeling off, the placenta with gauze is helpful. The uterus is then inverted, sutured with chromic catgut, and replaced in the pelvis. Several small doses (approximately 1 ml total) of Ergotrate (ergonovine maleate) are injected into different areas of the muscular wall to

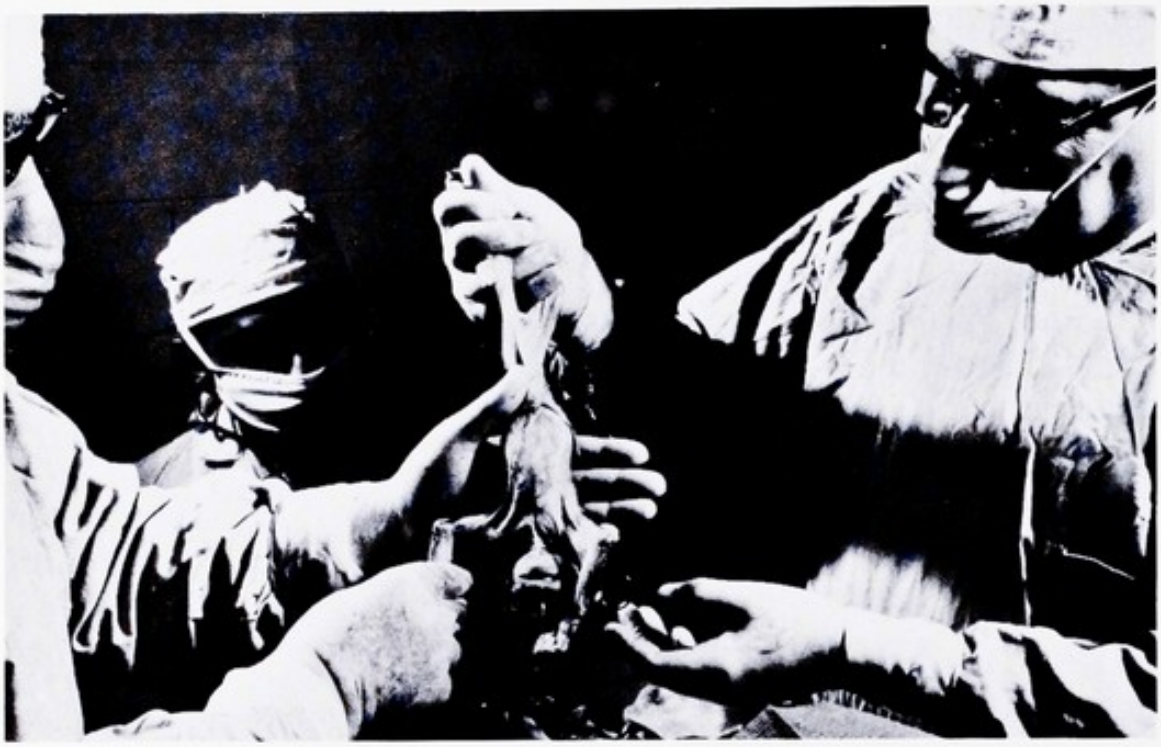

Fig. 23. Delivery of baby by cesarean section.

stimulate uterine contraction. Contraction can be aided by manual massage. Hemorrhage is seldom a problem with simians, and no attempt need be made to ligate vessels in the uterine wall. Inspection of the genital organs is made quickly before the abdominal wound is closed in layers. Interrupted, or continuous, nonabsorbable suture works well for skin closure. To prevent the animal from picking at the sutures, a subcuticular continuous catgut skin suture is used. No dressing is applied; the closed incision is sprayed with a plastic dressing (Rezifilm). If nonabsorbable skin sutures are used, they are removed 8–10 days postoperatively.

Repeated cesarean sections have been performed successfully. Usually, the incision through the abdomen and uterus is made at the locus of the scar tissue from the previous incision.

HERNIORRHAPHY

Herniorrhaphy is usually performed for umbilical hernia in simians. Occasionally ventral, incisional, and inguinal hernias must be repaired. The content of the hernial sac is usually adherent omentum

which can be lysed and replaced or removed. The procedure includes removal of the hernial sac and anatomical closure of the hernial defect.

FRACTURES

In the case of fractures, radiographic examination before and after reduction should be made. Fractures in simians are usually of the long bones of the extremities and result from rough handling. Closed reductions are splinted with plaster casts. In open reductions, Steinman intramedullary metal pins are removed following callus formation. Jaw fractures can be wired.

OTHER INJURIES

Eye enucleation and amputation of extremities, digits, and caudal segments are indicated as a result of severe trauma incurred when animals fight. Amputations of digits and tails are usually made by joint disarticulation, rather than by sectioning the bone. The guillotine-type operation is satisfactory with simians.

GASTROTOMY

Gastrotomy is performed when acute gastric dilatation cannot be relieved by intubation (Fig. 24).

SURGICAL DISRUPTIONS

Surgical disruptions are negligible. We have had only one in a baboon which picked at the nonabsorbable skin sutures.

RESEARCH PROCEDURES

Nephrectomy, splenectomy, thymectomy, hysterotomy, and orchidectomy have been performed for experimental or research purposes. In nephrectomy, a lumbar or transabdominal approach is used. For splenectomy, the incision most used is the oblique left subcostal. In a few instances in which other operations are combined—as in the wedge liver biopsy—an upper midline abdominal incision gives better

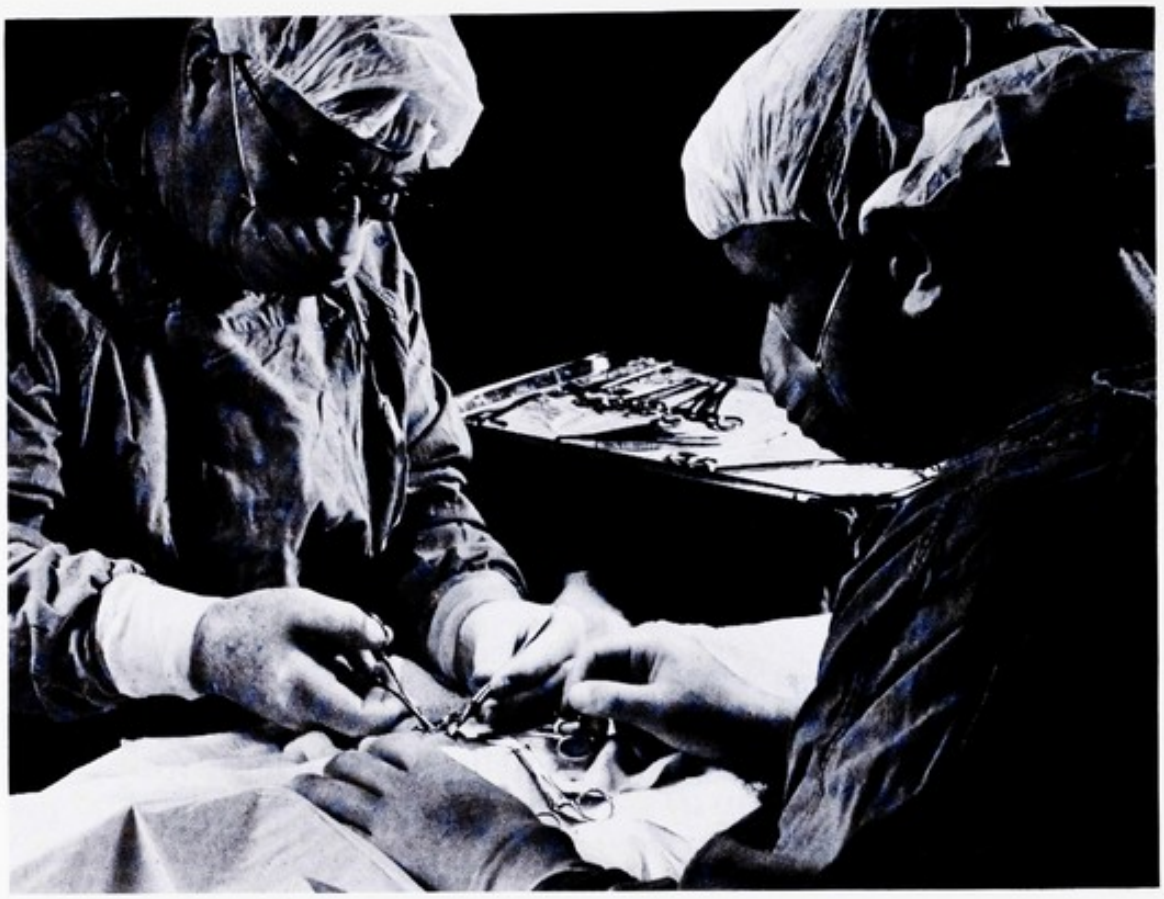

Fig. 24. Abdominal surgery.

exposure. Delivery of the stomach also delivers the spleen. Such a delivery can be eased by transecting the lienorenal ligament, which contains no blood vessels. The thoracotomy incision used to perform a thymectomy is an anterior midline sternum incision. Hysterotomy during pregnancy has been performed for various experimental purposes. Hysterotomy for examination of a fetus can be done at any stage of pregnancy. The optimum time for operation upon the fetus (most commonly in our experience, for purposes of thymectomy) is 85-105 days of gestation in the *M. mulatta*.

RECTAL AND VAGINAL PROLAPSE

Rectal and vaginal prolapse are fairly common in the simian. The former follows diarrhea and the latter occurs during or following pregnancy. The prolapse can be reduced by a gentle, milking manipula-

tion. A subcuticular nonabsorbable purse-string suture is placed around the orifice and tied not too tightly. The suture is removed in 5-7 days. Recurrences are few.

ABSCESSES

Incisions for drainage of abscesses are placed at dependent portions of the abscess, considering that the simian spends most of the time in an upright or sitting position. Drains are usually unnecessary, but if used should be sutured to the wound edges.

DILATATION AND CURETTAGE

Dilatation and curettage are indicated for retained secundines following an abortion or pregnancy. Palpation of the uterus with one hand during curettage is a helpful guide and minimizes accidental perforation of the uterus. If perforation should occur, immediate laparotomy with suture of the perforation in the uterus and administration of antibiotics is indicated. Removal of a dead fetus from the uterus by embryotomy techniques can be difficult, and if the fetus is over 85 days' gestation, it may require craniotomy and subsequent crushing of the fetal head. Curettage should be performed afterward.

TOOTH EXTRACTION

Extraction of the canine teeth in male simians makes handling of them safer. However, the teeth should not be removed before they have erupted through the gingiva. A mucoperiosteal flap is reflected from the tooth, downward on lower teeth, and upward on upper teeth, on both the lingual and labial sides. A scalpel is used for the gingival mucosa and a chisel for the periosteum. An automatic chisel with a variety of chisel points is satisfactory and the force and frequency of the blows can be adjusted. With the chisel, the tooth is loosened from its alveolar cavity and removed. The tooth should be completely removed; if the top breaks off, its root must be chiseled out. Other complications of teeth extraction are trauma to adjacent teeth, punctured accessory sinuses, and fractured jaws. Antibiotics are given for sinus puncture and fractured jaws are wired (Hilloowala and Miller, 1967).

RECORDS AND EQUIPMENT

Records are only as good and useful as they are adequately recorded and filed. Forms, specially designed for surgery and anesthesia are easily completed and more quickly used for later analysis. Our forms are made up in special pads in triplicate. One copy is kept in surgery, one is filed with the animal's protocol or record, and one is sent to the Data Processing Department. Record forms should be changed or modernized as the need arises.

Regular review of the records and reports by the surgical staff will keep personnel informed of the volume and quality of surgery and the morbidity and mortality rates.

While equipment and instruments need not be elaborate, there should be sufficient supply to carry through a busy day of surgery without having to clean, resterilize, and repackage instruments.

Careful, frequent, and systematic checking of equipment and instruments is necessary to effect needed repairs and replacements.

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APPENDIX I

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APPENDIX II

MANUFACTURERS AND SOURCES OF SUPPLIES

The following list is presented for the information of the reader. The concerns listed are not sole sources of supply or manufacture. The list has been compiled in an effort to be helpful and does not represent an endorsement of any particular product or manufacturer. We have generally found these sources quite adequate.

For further information regarding any product discussed in this guide, the reader should contact the referenced company.

Food Supplies

Alacta	Mead Johnson & Company 2402 West Pennsylvania Street Evansville, Indiana 47721
Baby food (cereal, fruit)	Gerber Products Company 445 State Street Fremont, Michigan 49412
Dextri-Maltose	Mead Johnson & Company 2402 West Pennsylvania Street Evansville, Indiana 47721
Duo-C.V.P capsules (vitamins)	USV Pharmaceutical Corporation 800 Second Avenue New York, New York 10017
Folic acid (in vitamin sandwich)	Fallek Products Company, Inc. 4 West 58th Street New York, New York 10021

Monkey chow	Ralston Purina Checkerboard Square St. Louis, Missouri 63109 Wayne Feed Supply Company, Inc. Box 86 Gaithersburg, Maryland 20760
Pervinal syrup (vitamins)	USV Pharmaceutical Corporation 800 Second Avenue New York, New York 10017
Similac with iron (baby formula)	Ross Laboratories 625 Cleveland Avenue Columbus, Ohio 43216
Sustagen (nutriment)	Mead Johnson & Company 2402 West Pennsylvania Street Evansville, Indiana 47721
Vi-Daylin (vitamins)	Abbott Laboratories 14th Street and Sheridan Road North Chicago, Illinois 60064

Pharmaceutical and Medical Supplies

Alconox (disinfectant)	Alconox Incorporated 215 Park Avenue South New York, New York 10017
Ambex (electrolyte formula)	Corvel 640 South Alabama Street Indianapolis, Indiana 46206
Atropine (antispasmodic)	Eli Lilly and Company 740 South Alabama Street Indianapolis, Indiana 46206
Bactrovet (antibiotic)	Pitman-Moore Division of the Dow Chemical Company P. O. Box 1656 Indianapolis, Indiana 46206
Biosol (antibiotic)	The Upjohn Company 7000 Portage Road Kalamazoo, Michigan 49002

Chloromycetin (antibiotic)	Parke, Davis & Company Joseph Campau at the River Detroit, Michigan 48232
Chlor-Trimeton (antihistamine)	Schering Corporation 1011 Morris Avenue Union, New Jersey 07083
Chymar (enzyme)	Armour-Baldwin Laboratories Box 3113 Omaha, Nebraska 68103
Clomiphene citrate (fertility drug)	The William S. Merrell Company Division of Richardson-Merrell, Inc. 110 East Amity Road Lockland Station Cincinnati, Ohio 45215
Coferrin	Ulmer Pharmacal Company 1400 Harmon Place Minneapolis, Minnesota 55403
Cosa-terramycin (antibiotic)	Chas. Pfizer & Company, Inc. 235 East 42nd Street New York, New York 10017
Delalutin (progestational hormone)	E. R. Squibb and Sons 745 Fifth Avenue New York, New York 10022
Dextrose solution, parenteral	Cutter Laboratories 4th and Parker Streets Berkeley, California 94710
Dihydrostreptomycin (antibiotic)	Chas. Pfizer & Company, Inc. 235 East 42nd Street New York, New York 10017
Diryl (ectoparasite dust)	Pitman-Moore Division of the Dow Chemical Company P. O. Box 1656 Indianapolis, Indiana 46206
Dopram (analeptic)	A. H. Robins Company, Inc. 1407 Cummings Drive Richmond, Virginia 23220

Elase (enzyme)	Parke, Davis & Company Joseph Campau at the River Detroit, Michigan 48232
Ergotrate (to stimulate uterine contractions)	Eli Lilly and Company Box 618 Indianapolis, Indiana 46206
Erythromycin (antibiotic)	Abbott Laboratories 14th Street and Sheridan Road North Chicago, Illinois 60064
Furoxone (antibiotic)	Eaton Laboratories Division of the Norwich Pharmacal Co. 17 Eaton Avenue Norwich, New York 13815
Hi-Septic (acid cleaning solution)	Center Chemical Company P. O. Box 1888 5001 Peachtree Boulevard Atlanta, Georgia 30301
Innovar-Vet (anesthetic)	McNeil Laboratories, Inc. Camp Hill Road Fort Washington, Pa. 19034
Isoniazid (antituberculosis drug)	Panray-Parlam Corporation 223 South Dean Street Englewood, New Jersey 07631
Kantrex (antibiotic)	Bristol Laboratories Division of Bristol-Myers Company P. O. Box 657 Syracuse, New York 13201
Kaopectate (antidiarrheal)	The Upjohn Company 7000 Portage Road Kalamazoo, Michigan 49002
Keflin (antibiotic)	Eli Lilly and Company Box 618 Indianapolis, Indiana 46206
Koagamin (antihemorrhagic)	Chatham Pharmaceuticals, Inc. 901 Broad Street Newark, New Jersey 07102

Lincocin (antibiotic)	The Upjohn Company 7000 Portage Road Kalamazoo, Michigan 49002
Liquamycin (antibiotic)	Chas. Pfizer & Company, Inc. 235 East 42nd Street New York, New York 10017
Longicil (antibiotic)	Fort Dodge Laboratories Warden Building P. O. Box 518 Fort Dodge, Iowa 50501
Metofane (anesthetic)	Pitman-Moore Division of the Dow Chemical Co. P. O. Box 1656 Indianapolis, Indiana 46206
Mycostatin (antifungal agent)	E. R. Squibb and Sons 745 Fifth Avenue New York, New York 10022
Nembutal (anesthetic)	Abbott Laboratories 14th Street and Sheridan Road North Chicago, Illinois 60064
NegGram (antibiotic)	Winthrop Laboratories 90 Park Avenue New York, New York 10016
Neo-Decadron (antihistamine cream)	Merck, Sharpe & Dohme Division of Merck & Company, Inc. West Point, Pennsylvania 19486
Neo-Delta-Cortef ointment (antihistamine)	The Upjohn Company 7000 Portage Road Kalamazoo, Michigan 49002
Nolvasan (liquid disinfectant)	Fort Dodge Laboratories Warden Building P. O. Box 518 Fort Dodge, Iowa 50501
Nonemic (hematinic)	Armour-Baldwin Laboratories Box 3113 Omaha, Nebraska 68103

Paregoric (antispasmodic)	Eli Lilly and Company Box 618 Indianapolis, Indiana 46206
Pentothal Sodium (anesthetic)	Abbott Laboratories 14th Street and Sheridan Road North Chicago, Illinois 60064
Pepto-Bismol (gastric antacid)	The Norwich Pharmacal Co. 17 Eaton Avenue Norwich, New York 13815
Phenocide (bactericidal detergent)	Center Chemical Company P. O. Box 1888 5001 Peachtree Boulevard Atlanta, Georgia 30301
pHisoHex (germicidal soap)	Winthrop Laboratories 90 Park Avenue New York, New York 10016
Polycillin N (antibiotic)	Bristol Laboratories Division of Bristol-Myers Company P. O. Box 657 Syracuse, New York 13201
Polymixin B (antibiotic)	Chas. Pfizer & Company, Inc. 235 East 42nd Street New York, New York 10017
Rezifilm (plastic dressing)	E. R. Squibb and Sons 745 Fifth Avenue New York, New York 10022
Ringer's Solution (plasma expander)	Cutter Laboratories 4th and Parker Streets Berkeley, California 94710
Sernylan (tranquilizer)	Parke, Davis & Company Joseph Campau at the River Detroit, Michigan 48232
Thibenzole (anthelmintic)	Merck, Sharpe & Dohme Division of Merck & Company, Inc. West Point, Pennsylvania 19486

Thorazine (antiemetic)	Smith Kline & French Laboratories 1500 Spring Garden Street Philadelphia, Pa. 19101
Tranvet (tranquilizer)	Diamond Laboratories, Inc. 2538 S. E. 43rd Street Des Moines, Iowa 50317
Tuberculin, Old	Parke, Davis & Company Joseph Campau at the River Detroit, Michigan 48232
Tylocine (antibiotic)	Corvel 640 South Alabama Street Indianapolis, Indiana 46206
Unipen (antibiotic)	Wyeth Laboratories, Inc. P. O. Box 8299 Philadelphia, Pa. 19101
Vetafil (sutures)	Bengen Manufacturing Company 812 Dreyerstrasse Hanover, West Germany
Zephiran, tincture of (disinfectant)	Winthrop Laboratories 90 Park Avenue New York, New York 10016

Mechanical and Support Service Equipment

Air filters (high efficiency)	Cambridge Filter Corporation P. O. Box 1255 Syracuse, New York 13201
	Flanders Filters, Inc. P. O. Box 178 Riverhead, New York 11901
Cage washers	R. G. Wright Company, Inc. 2280 Niagra Street Buffalo, New York 14207
Dryers, laundry (commercial)	Wm. Cissell Manufacturing Co. 831 South First Street Louisville, Kentucky 40203

Incinerator	Brule Incinerator 13920 Western Avenue Blue Island, Illinois 60406
Washing machines (commercial)	Speed Queen Division of McGraw Edison Co. Ripon, Wisconsin
	Troy Launderite Division of Ametck, Inc. East Moline, Illinois 61244
Waste grinders	In-Sink-Erator Manufacturing Co. 1225 14th Street Racine, Wisconsin 53403

Construction Materials

Dex-O-tex-Neotex (floor covering)	Crossfield Products Corporation 140 Valley Road Roselle Park, New Jersey 07204
Liquid Tile (wall covering)	Evershield Products Company Joppa, Maryland 21085

Laboratory Apparatus and Miscellaneous Supplies

Baby bottles	Pyramid Rubber Company 771 North Freedom Street Ravenna, Ohio 44266
Ethaire anesthesia resuscitation units	Air-Shields, Inc. Hatboro, Pennsylvania 19040
Autodilutor	Scientific Products 1210 Leon Place Evanston, Illinois 60201
Electronic cell counter (Coulter Model B)	Coulter Electronics 590 West 20th Street Hialeah, Florida 33010
Disposable tubes, needles, Sedi-Rack, syringes, Vacutainers, Unopette	Becton, Dickinson & Company Cornelia Street East Rutherford, New Jersey 07073

Restraint chair	Foringer Company, Inc. 535-A Southlawn Lane Rockville, Maryland 20850
Resuscitator, bassinet (Dann)	Ohio Chemical & Surgical Co. Div. of Air Reduction Company, Inc. 1400 E. Washington Avenue Madison, Wisconsin 53703
Sterility indicator (Diack)	Smith and Underwood 1847 North Main Street Royal Oak, Michigan 48073
Still	Barnstead Still & Sterilizer Co. Two Lanesville Terrace Boston, Massachusetts 02131
	Bellco Glass, Inc. Box B Vineland, New Jersey 08360
Stimulator, Model SD5 (electroejaculator)	Grass Instrument Company 101 Old Colony Avenue Quincy, Massachusetts 02169
X-ray unit, film, and supplies	Picker X-Ray 1275 Mamaroneck Avenue White Plains, New York 10605

Housing Equipment

Automatic drinking water device (Lixit)	ATCO Manufacturing Company 1106 Hardman Avenue Napa, California 94558
Automatic drinking water device (Hardco)	Hardco Scientific 6811 Grace Avenue Cincinnati, Ohio 45215
Cage (adult male and female)	Harford Metal Products, Inc. Aberdeen, Maryland 21001
Cage and enclosure (Landon unit)	Harford Engineering Company Otsego & Juniata Streets Havre de Grace, Maryland 21078

Cage and enclosure (Kirschner)	Kirschner Manufacturing Company Vashon, Washington 98070
Filter media, isolator enclosures (FG-50)	American Air Filter Company, Inc. 215 Central Avenue Louisville, Kentucky 40208
Incubator, pediatric (Bunn's Baby Haven)	John Bunn Corporation 11035 Walden Avenue Alden, New York 14004
Isolator enclosure	Matthews Research, Inc. 4306 Wheeler Avenue Alexandria, Virginia 22304
Isolette	Harford Engineering Company Otsego and Juniata Streets Havre de Grace, Maryland 21078
Rack, cage	Harford Metal Products, Inc. Aberdeen, Maryland 21001

SUBJECT INDEX

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