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Mouse Breeding and Husbandry

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Simply inoculating viable *Mycobacterium leprae* into foot pads of mice is not sufficient to ensure multiplication of the organisms. Among other requirements, it is necessary that the mice be "clean," uniform, and of a suitable strain. The effects of environmental and genetic factors on multiplication of *M. leprae* in the food pad of the immunologically normal mouse have not, on the whole, been systematically studied. Most reports, particularly those on environmental factors such as intercurrent infections or nutrition, are anecdotal. Therefore, conclusive evidence of an effect of these factors on multiplication of *M. leprae* may not exist. On the other hand, it is clear that a continuing supply of mice of good quality—that is, free of certain infections, uniform, and of a strain that sustains multiplication of the organisms—is essential to the success of a laboratory dedicated to the mouse foot-pad technique. As a consequence of an increase of non-specific immunity, mice infected with certain pathogenic organisms may resist multiplication of *M. leprae,* whereas sick mice may not survive long enough to permit multiplication of the organisms. In addition, variation from mouse to mouse should be minimal. Finally, all other things being equal, strains of mice are known to vary in their ability to sustain multiplication of *M. leprae.*

Breeding

Mice suitable for inoculation with *M. leprae* are widely available from commercial breeders but, for reasons of economy, most laboratories breed their own. In a quantitative sense, mice breed readily. The duration of gestation is approximately 21 days, and the female is fertile immediately after parturition. The average productivity during the nine months or so of a female's active breeding life is of the order of six litters of six mice each. Thus, one can readily estimate his requirements, calculate the number of breeding pairs or "families" needed to produce these, and add a proportion to be used to replace the breeders. The most common practice is that of "harem-mating"; one male is placed together with two or three females. If one wishes to breed mice of good quality, however, the matter is somewhat more complicated than is suggested by these quantitative considerations. In order to be certain of a supply of clean mice, one must begin with a clean breeding nucleus. This is to say, one must obtain the clean mice for a nucleus from an appropriate outside source.

A prime consideration in selecting the breeding system to be used is the uniformity of the mice. Ideally, mouse-to-mouse variation of multiplication of *M. leprae* should reflect variation among inocula, and not individual variation among mice. One method of obtaining uniform mice is *inbreeding.* An inbred colony of mice is one in which the maximal degree of genetic identity among individuals has been achieved by brother-sister or parent-offspring matings. After 20 generations (about six years are required), the mice have become virtually homozygous—i.e., genetically identical. A *random-bred* colony is one in which genetic variation is maintained and uniformly distributed throughout the colony. Although random-bred mice are generally more robust and more fertile than are inbred mice, the more uniform character of inbred mice makes them more suitable for research. In addition, random-bred colonies must be very large, in order to avoid inadvertent inbreeding; this may be disadvantageous, if the required numbers of experimental mice are small. Finally, the investigator who decides in favor of an inbred colony must pay attention to a practical problem. Because the breeding nucleus will necessarily represent a very small fraction of the parent colony from which the nucleus was derived, random mutations may accumulate and, in

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the course of many generations, cause the local strain to diverge genetically from its parent strain.

One begins an inbred colony by obtaining a nucleus of 10–20 pairs from a reliable source. The offspring produced by these pairs are brother-sister mated in permanent families. Reliable methods for identifying animals—e.g., labeling of cages and punching of ears—should be employed, and good pedigree records should be maintained. To keep sublines short, all animals in the colony at any one time should be traceable to one family established not more than five generations earlier; sublines should be selected for productivity by eliminating the least fertile. From time to time, if possible, animals should be tested for histocompatibility, by means of skin grafting and mixed lymphocyte reactions. To prevent genetic drift away from the parent colony, it is wise, from time to time, to obtain new breeding stock from the original source, to be used as a new breeding nucleus.

Choice of inbred strain

A large number of inbred mouse strains have been developed, some of which have been tested for their ability to sustain multiplication of *M. leprae.* Certain strains have been found more suitable than others ([6]). On the other hand, such differences aş exist among inbred strains are small, and not nearly as important as the strain differences reported for susceptibility to *M. lepraemurium* ([1]) and *Leishmania* ssp. ([4]). Recent studies ([2]) have suggested that, following immunization with *M. leprae,* the lymphoproliferative response varies among inbred strains of mice; however, whether this variation correlates with differences among strains with respect to multiplication of *M. leprae* in the foot pad is not clear. The two strains of inbred mice that have been most widely and successfully used are BALB/c and CBA.

Husbandry

Housing. If one obtains a breeding nucleus of clean mice of one of these two strains, and can maintain the mice and their progeny free of certain pathogenic organisms, he is "in business." However, great care must be taken to prevent infection of the colony with pathogenic organisms. The most likely source of infection is contamination from wild rodents, either by direct contact, or by contamination of diet or bedding. The mice should be housed in clean, vermin-proof quarters. To exclude vermin to the maximal degree possible, the animal quarters must be fully enclosed. In order to expedite cleaning, the mouse cages should be placed above the floor, on fixed shelves or movable racks; the interior of the building in which the animals are housed should be finished; and there should be an abundant supply of clean, running water.

The breeding colony and the mice infected with *M. leprae* should be housed in separate rooms. Mice inoculated with *M. leprae* may be caged, separated by sex, in groups of five to ten, depending upon the size of the cage; a commonly used cage suitable for housing five mice is 15 cm wide, 15 cm high, and 30 cm long (6 × 6 × 12 inches). Avoidance of crowding aids breeding, and minimizes the needed frequency of cleaning. Cages of seamless construction, ideally fabricated from stainless steel or heat-resistant plastic (polycarbonate), are easily cleaned. However, cages fabricated locally from aluminum or galvanized metal are certainly suitable. Cage lids may similarly be locally fabricated from stainless or galvanized wire mesh. Shelves and racks may also be locally fabricated—from galvanized metal or laminate-on-wood (i.e., Formica®)—to permit easy cleaning.

A final consideration is the cleanliness of bedding and water. It makes little sense to provide, at great expense, animal quarters from which vermin are excluded, and at the same time to use vermin-infested bedding. Any number of locally available materials may be used as bedding—wood shavings, sawdust, ground corn cobs, chopped straw or hay, shredded paper or rice husks, for example. Ideally, the bedding should be autoclaved shortly before use, especially if it is stored in bulk for any length of time prior to use. A layer of bedding 2–3 cm in depth should be employed. The water should be free of *Salmonella* ssp. and other enteric organisms; if a source of clean water cannot be depended upon, chlorination (1–3 mg per l or parts per million as Cl_2) or autoclaving should be considered. Addition of HCl to a

TABLE 1. *Nutritious diets for the laboratory mouse.**

	Composition (g per kg diet)			
	Diet 1	Diet 2	Diet 3	Diet 4
A. Ingredients				
Ground milling wheat	515.00	329.50	560.00	602.00
Nonfat skim milk, edible	200.00	120.00	200.00	200.00
50% dehulled soybean meal	112.50	67.50	112.50	25.00
Corn oil, edible grade	102.50	34.50	57.50	102.50
Dried brewer's yeast	40.00	24.00	40.00	40.00
Sodium chloride	13.75	8.75	13.75	13.75
Dicalcium phosphate	10.00	10.00	10.00	10.00
Ferric citrate	1.25	0.75	1.25	1.25
Vitamin premix (calculated from C, below)	5.00	5.00	5.00	5.00
Wheat germ meal, edible		400.00		
B. Components				
Protein	21.2	24.9	21.3	17.9
Fat	10.9	8.2	7.1	11.2
Fiber	1.7	2.1	1.1	1.1
Ash	4.7	4.7	4.8	4.3
Ca	1.4	0.9	1.0	0.9
P	0.9	1.1	0.9	0.8
C. Vitamins	Recommended daily intake per kg feed			
A				
E (α-tocopherol)		242–5,500 IU		
B_{12}		9.9–27 mg		
Riboflavin		3.9–5.5 mg		
Niacin		2.4–11 mg		
Pantothenic acid		26.4–143 mg		
Choline		9.9–55 mg		
Folic acid		495–1,452 mg		
Menadione (vitamin K activity)		560–2,750 mg		
Pyridoxine (B_6)		0.99–5.5 mg		
Biotin		19.8–165 mg		
D		143–5,060 IU		
Thiamine		2.2–9.9 mg		

* Adapted from reference no. 3.

final concentration of 0.003–0.006 N helps to minimize bacterial contamination of the water. Water is usually administered in a sterilizable bottle of 500 ml capacity, equipped with a one-hole rubber stopper containing a metal (stainless steel) sipper tube.

Apart from intercurrent infection, the most important environmental factor is temperature. Ideally, the animal quarters should be maintained at a temperature of 20–25°C. Not only do *M. leprae* multiply better in mice housed at this temperature ([5]), but both the health and fertility of the animals are improved. The humidity should be maintained constant at 80%.

Nutrition. Mice require a diet composed of 20% protein, 10% fat, various minerals, vitamins and unsaturated fats. A nutritious diet is shown in Table 1. Locally available components should be substituted where possible. A laboratory mouse requires 3–5 g diet daily, on average.

The diet can be provided in the form of pellets or mash. Administration of pellets is more convenient; these are placed in a depression in the cage lid, or in a feeder suspended inside the cage. A mash diet possesses an obvious advantage, if a drug is to be incorporated. However, the mash must be placed in a container inside the cage, and is therefore more easily fouled and wasted by the mice. Because some components—especially vitamins—are unstable, the diet should be used within 30 days of manufacture, unless it can be stored in the cold.

During storage, the diet must be protected from vermin. Finally, the diet must be free of contamination by *Salmonella* sp., common contaminants of foodstuffs, especially in the tropics.

Care. Not only must the mice be free of contamination by pathogenic bacteria, viruses, and parasites when the mouse colony is first established, but the colony must also be maintained free of contamination. The most effective measures in maintaining a colony of disease-free mice are a high standard of cleanliness and the observance of a few simple precautions.

Cleaning cages—as a minimum, replacing the soiled bedding—must be carried out once or twice weekly, depending upon the number of mice per cage. At least as frequently, water bottles must be exchanged. In addition, the cages should be inspected daily, to ensure adequate supplies of food and water. Periodically, the cages should be washed and, if possible, autoclaved. As a minimum, this must be done before a cage is used to house a new group of mice.

In addition to cleanliness of cages, food and water supply, cleanliness of the surroundings must also be maintained. Mouse colonies produce a considerable quantity of litter, both that generated by the mice, and that created in caring for them—e.g., during cage cleaning, and as a result of transferring feed from storage container to cage. The litter must not be permitted to accumulate, but should be removed periodically by sweeping and washing the shelves and floor.

Animal handlers should be provided with facilities for handwashing and several changes of clothing to be worn only in the animal quarters, and they should be required to use them. The clothing should be donned upon first entering the animal quarters in the morning, taken off upon leaving at the end of the work day, and laundered frequently.

As a precaution against introducing a new pathogenic organism, it is essential that animals from another source not be introduced into the animal quarters.

Finally, cages must be clearly and unambiguously labeled. If the same cage is employed continuously, the label should be fixed to the cage by an adhesive tape that is resistant to wetting. If, on the other hand, cages are frequently exchanged, the label should take the form of a stiff card, and the cages must be fitted with card holders that permit ready insertion and removal of the card.

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