

Chinese hamsters (*Cricetulus griseus*) are of interest to cytogeneticists because their chromosomes are rather easy to study (Brooks et al., 1973). Several outbred, inbred, and mutant stocks have been developed, but they are not as readily available as some other rodents. The life span characteristics of this species have not been rigorously investigated; however, although typical survival curves have been demonstrated for females, the curves for males, which usually live longer, are atypical. An MxLS of about 45-50 months has been reported for males (Benjamin and Brooks, 1977). Information on pathology is available for the colony maintained at the Lovelace Foundation Inhalation Toxicology Research Institute, Albuquerque, New Mexico (Benjamin and Brooks, 1977). Husbandry and dietary requirements have been discussed in [Chapter 5](#).

### **Gerbils**

Cheal (1986) has provided a comprehensive review of the Mongolian gerbil (*Meriones unguiculatus*) as a model for research on aging and has concluded that its ease of handling, ready availability, and particular physiologic and behavioral attributes establish it as a valuable model system. However, the gerbil exhibits an atypical survival curve ([Figure 8.3](#)), and much more must be learned about the causes for this, including susceptibility to various infectious diseases and nutritional requirements. All gerbils in the United States are descended from only nine animals (Cheal, 1986), and there is some concern that deleterious recessive or dominant mutations might have become fixed in the population (M. Cheal, University of Dayton Research Institute, Higley, Arizona, unpublished). The husbandry of gerbils is discussed in [Chapter 5](#).

## **RODENT MODELS OF INSULIN-DEPENDENT DIABETES MELLITUS**

With rare exceptions, the rat and mouse models of human autoimmune diabetes mellitus have appeared spontaneously, presumably as a result of mutation, rather than deliberate genetic manipulation. The discussion below focuses on two models of insulin-dependent diabetes mellitus: the BB rat and the NOD mouse. The management principles suggested are easily superimposed on standard rodent-management techniques.

### **Diabetes-Prone and Diabetes-Resistant Rats**

In 1974, some animals were found in a closed colony of outbred WI rats (Bio-Breeding Labs, Ottawa, Ontario) that spontaneously developed autoimmune diabetes mellitus (Chappel and Chappel, 1983). Several inbred diabetes-prone and diabetes-resistant strains were developed from this outbred

stock at the Department of Pathology, University of Massachusetts Medical School. The diabetes-prone strains are designated BBBA/Wor, BBDP/Wor, BBBE/Wor, BBNB/Wor, and BBPA/Wor; the diabetes-resistant strains are designated BBDR/Wor and BBVB/Wor.<sup>1</sup> The genetics and pathophysiology of the diabetes-prone strains have been reviewed (Guberski, 1993; NRC, 1989).

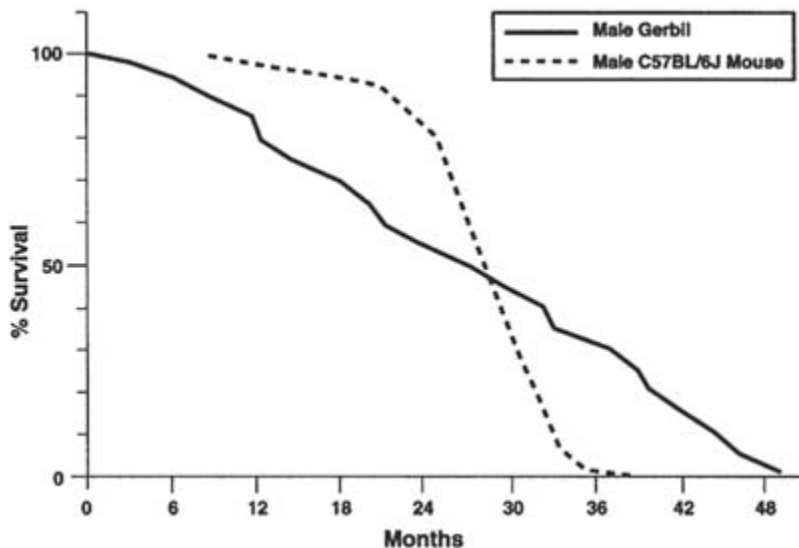


FIGURE 8.3 Survival of conventionally reared male Mongolian gerbils. From Cheal (1986).

### Breeding Techniques and Genetic Records

Foundation colonies of diabetes-prone and -resistant strains are maintained strictly by full-sib matings. However, the selection of litters from which future generations of breeders will be derived is influenced by the presence of desired phenotypic traits (e.g., incidence of diabetes, age at onset of diabetes, fertility, litter size, and survival of pups to weaning). Although it is recognized that the imposition of selection criteria can delay achieving inbred status, the goals of any breeding strategy must include preservation of the desired phenotypic characteristics (e.g., the development of diabetes mellitus).

<sup>1</sup> The designation BB/Wor was originally used as a group name for all seven inbred strains.

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Essential data on each litter produced in the foundation colonies must be recorded to permit genetic tracing of breeding stock from one generation to another. To achieve this, a system of identification of each member of the primary and secondary breeding branches must be established. The records should include the occurrence of phenotypic characteristics, such as diabetes, thyroiditis, and lymphopenia.

### Husbandry and Care

It is desirable that diabetes-prone and -resistant rats be maintained free of rodent pathogens in appropriate barrier facilities (see [Chapter 5](#)) because of the effect of these pathogens on phenotypic expression of diabetes (reviewed by Guberski, 1993). Microbiologic status should be monitored and recorded; records should include the tests performed and the frequency of testing. Experience has shown that these animals do well on a conventional light:dark ratio of 12:12 hours.

*Detection and treatment of diabetes mellitus.* The most cost-effective method of screening for diabetes is to test for glycosuria. Urine is expressed from the bladder manually by gently compressing the bladder against the pubic symphysis. Urinary glucose concentration is measured with a glucose test strip. Positive urine tests are confirmed with blood glucose measurements. Blood samples should be obtained from the tail within 2 hours of the urine test and tested with an appropriate technique. Animals testing 4+ for glycosuria and having blood glucose concentrations greater than 250 mg/dL are considered diabetic.

The age at which to begin testing and the frequency of testing for diabetes depend on the unique characteristics of the particular model and the environmental conditions under which it is kept. Testing for glycosuria should be started before the expected onset of diabetes and performed at least three times per week at the start of the light period in the light-dark cycles. The frequency of glycosuria testing can be reduced after about 120 days because new occurrences are less likely.

Daily treatment of diabetic rats with insulin is mandatory and should begin on the day that glycosuria is found and diabetes is confirmed. The daily dose of insulin will be a function of age, body weight, the presence of ketoacidosis and dehydration, and the presence of pregnancy or lactation. [Table 8.2](#) provides guidelines for the initial doses of insulin for animals that become diabetic after the age of 65 days. Animals that become diabetic *on or before the age of 65 days* should receive 0.2 U of insulin per 100 g of body weight in addition to the dose indicated. As animals increase in weight, the dose of insulin is increased by 0.2 U/10 g of body weight if the animals became diabetic on or before the age of 65 days, and by 0.2 U/16 g

of body weight if the animals became diabetic after the age of 65 days. The maximal daily dose should not exceed 1.4 U/100 g of body weight for animals that became diabetic on or before 65 days of age, and 1.25 U/100 g of body weight for animals that became diabetic after the age of 65 days.

If ketonuria (as detected with a test strip) develops, the dose of insulin should be increased, and lactated Ringer's solution with sodium bicarbonate should be administered in the amounts shown in [Table 8.3](#). Injections of fluids are well tolerated when given under the loose skin on the back (distal to the nape of the neck).

TABLE 8.2 Starting Doses of Insulin for BB/Wor Rats That Become Diabetic After the Age of 65 Days

	Initial Blood Glucose Concentration, mg/dL					
	250	300	350	400	450	500+
Body weight, g <sup>a</sup>	Starting Dose of Insulin, <sup>b</sup> U					
100	0.4	0.6	0.6	0.6	0.8	0.8
125	0.4	0.6	0.6	0.8	0.8	0.8
150	0.6	0.8	0.8	1.0	1.0	1.2
175	0.8	1.0	1.0	1.2	1.2	1.4
200	1.0	1.2	1.2	1.4	1.6	1.6
225	1.2	1.4	1.4	1.6	1.6	1.8
250	1.4	1.6	1.6	1.8	1.8	2.0
275	1.4	1.6	1.8	1.8	2.0	2.0
300	1.4	1.6	1.8	2.0	2.0	2.2
325	1.6	1.8	2.0	2.0	2.2	2.2
350	1.6	1.8	2.0	2.2	2.2	2.4
375	1.8	2.0	2.2	2.2	2.4	2.4
400	2.0	2.2	2.4	2.4	2.6	2.6
425	2.2	2.4	2.6	2.6	2.8	3.0
450	2.2	2.4	2.6	2.8	3.0	3.2

<sup>a</sup> Assumes that rat is well hydrated and that ketosis, if present, is being corrected.

<sup>b</sup> PZI U40 (Eli Lilly) insulin and a U/100 Lo-dose syringe (B-D) are used. U40 insulin + U/100 syringe = 0.4 units per gradation mark. Add 0.2 U/100 g of body weight to the dose for animals that develop diabetes on or before the age 65 days. Maximal daily dose equals 1.4 U/100 g of body weight for animals that become diabetic on or before the age of 65 days and 1.25U/100 g of body weight for animals that become diabetic after the age of 65 days.

*Treatment of hypoglycemia.* Hypoglycemia is defined as severe if blood glucose is less than 40 mg/dL, moderate if blood glucose is 40-60 mg/dL, and mild if blood glucose is 60-80 mg/dL. The successful treatment of hypoglycemia requires a decrease in insulin dose combined with subcutaneous injections of fluid. Suggested regimens are outlined in [Table 8.3](#).

*Care of pregnant females.* If pregnant animals become aglycosuric, the course of action depends on the ratio of insulin to "ideal" body weight

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(IBW). The IBW of a pregnant female at the age of 90 days is considered to be 270 g. If the animal is more than 90 days old, the body weight of a nonpregnant female sibling should be used as the IBW. The following procedures are recommended:

TABLE 8.3 Treatment for Ketonuria in BB/Wor Rats

Ketones	Increased Insulin, <sup>a</sup> U/100 g body wt	Lactated Ringer's Solution, cm <sup>3</sup>	Sodium Bicarbonate, mEq <sup>b</sup>
2+	0.2	10.0	0.0
3+	0.2	9.0	1.0
4+	0.2	18.0	2.0

<sup>a</sup> Insulin dose of lactating females should not exceed 1.0 U/100 g of "ideal" body weight (see Care of pregnant females). Dose should not be increased during mild episodes of ketonuria.

<sup>b</sup> 1 cm<sup>3</sup> of 8.4% sodium bicarbonate equals 1 mEq.

SOURCE: Guberski, 1993.

- If the ratio of insulin to IBW is greater than 1.0 U/100 g, the dose of insulin should be reduced by 15 percent.
- If the ratio of insulin to IBW is 0.9-1.0 U/100 g, the dose of insulin should be reduced by 10 percent and 10 cm<sup>3</sup> of lactated Ringer's solution should be administered.
- If the ratio of insulin to IBW ratio is less than 0.9 U/100 g, the dose of insulin should be reduced by 0.2 U/100 g and 10 cm<sup>3</sup> lactated Ringers solution should be administered.

If pregnant animals are severely hypoglycemic, follow the instructions for treating hypoglycemia in [Table 8.4](#).

If a female becomes ketotic at parturition, the insulin dose should not be changed. Instead, lactated Ringer's solution and sodium bicarbonate should be injected subcutaneously in the amounts indicated in [Table 8.3](#).

*Care of lactating females.* Beginning 12-14 days after delivery, insulin should be decreased by 10-15 percent each day until a dose of 0.8-1.0 U/100 g of IBW is achieved. To prevent hypoglycemia in lactating females, food should be made readily accessible by placing it on the cage floors. If hypoglycemia occurs, it should be treated as indicated in [Table 8.4](#).

### Use of Spleen Cells to Reduce Frequency of Diabetes and Improve Breeding Efficiency

Diabetes-prone rat strains are profoundly T-cell lymphopenic. Injections of neonatal bone marrow, fresh spleen cells, or concanavalin-A-stimulated

spleen cells correct the T-cell lymphopenia and substantially reduce the frequency of spontaneous diabetes (Naji et al., 1981; Rossini et al., 1984). Fresh spleen cells are obtained from diabetes-resistant rats, which are histocompatible with diabetes-prone rats but are not lymphopenic. Spleens are prepared with standard techniques (Burststein et al., 1989). Diabetes prone rats between 21 and 40 days old receive one spleen equivalent of fresh donor cells in 1 cm<sup>3</sup> of RPMI medium 1640, administered intraperitoneally. This procedure reduces the incidence of diabetes from greater than 85 percent to about 15 percent. Nondiabetic females do not require daily insulin injections (this reduces the workload of the staff) and are more productive breeders, as shown in [Table 8.5](#).

TABLE 8.4 Treatment for Hypoglycemia in Diabetic BB/Wor Rats

Classification (blood glucose concentration)	Subcutaneous Fluid Therapy	Change in Insulin Dose	Change in Time of Insulin Administration
Severe (<40 mg/dL)	Give 1 cm <sup>3</sup> 50% dextrose; 2 hrs later give lactated Ringer's solution with 5% dextrose	Reduce by 30-50%	Delay by 2-3 hrs
Moderate (40-60 mg/dL)	Give 10 cm <sup>3</sup> lactated Ringer's solution with 5% dextrose	Reduce by 20-30%	Delay by 2-3 hrs
Mild (60-80 mg/dL)	Give 10 cm <sup>3</sup> lactated Ringer's solution	Reduce by 10-15%	No delay

SOURCE: Guberski, 1993.

### Shipping Pathogen-Free Rats

Diabetes-prone rats have severely compromised immune systems and should be shipped in crates designed to keep them free of rodent pathogens (see [Chapter 6](#)). Drinking water or a water-rich material must be provided, especially for diabetic rats showing signs of polydipsia and polyuria, because these animals are prone to dehydration. Commercial carriers should be instructed to use climate-controlled trucks and holding rooms because diabetic rats are more susceptible than normal rats to fluctuations in temperature. In addition, commercial carriers must guarantee delivery within 24 hours because shipping delays are hazardous for animals that require daily insulin injections.

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TABLE 8.5 Reproduction in Diabetes-Prone BB/Wor Rats Before and After Receiving Splenocytes from Diabetes-Resistant BB/Wor Rats

	Diabetes-Prone Females Not Treated with Splenocytes (N = 1,238)	Diabetes-Prone Females Treated with Splenocytes (N = 1,022)
Incidence of diabetes	86%	16%
No. pups born	7,160	12,434
No. pups weaned	5,766	10,918
Pup survival through weaning	80.5%	87.8%
No. pups weaned per female mated	4.7	10.7

SOURCE: Guberski, 1993.

### NOD Mice

NOD (nonobese diabetic) is an inbred strain derived from Jcl:ICR mice with selection for the spontaneous development of insulin-dependent diabetes (Makino et al., 1980). The expression of diabetes in this strain is under polygenic control (Leiter, 1993). Clinical features of diabetes in NOD mice are similar to those in humans. Females develop diabetes at a higher incidence and at an earlier age than males. The genetics and pathophysiology of this model have been reviewed (Leiter, 1993; NRC, 1989).

Insulin treatment is required to maintain diabetic NOD mice; without insulin, they survive only 1-2 months after diagnosis. Diabetes is diagnosed by determining that the blood (nonfasting) or plasma glucose concentration is increased. This determination can be made by measuring blood glucose directly or by measuring urinary glucose with a glucose test strip. Glycosuria, as read on the test strip, usually denotes a plasma glucose of 300 mg/dL. Large numbers of mice can be easily screened by this method.

It is difficult to keep serum glucose within a normal range with insulin treatment, but body weight can be maintained and life prolonged (Ohneda et al., 1984). Morning and evening intraperitoneal injections of a 1:1 mixture of regular and NPH insulin are satisfactory. The dose will be 1-3 U, depending on the extent of glycosuria.

Environmental factors are extremely important in the expression of diabetes in NOD mice. Keeping them in an SPF environment increases the occurrence of diabetes; exposure to a variety of murine viruses, including mouse hepatitis virus (Wilberz et al., 1991) and lymphocytic choriomeningitis virus (Oldstone, 1988), prevents diabetes development. That various types of exogenous immunomodulators prevent the development of diabetes (Leiter, 1990) suggests that infectious agents prevent diabetes by general immunostimulation. Diet also has an important effect on diabetes development: natural-ingredient

diets, including standard, commercially available mouse feed, promote a high incidence of diabetes (Coleman et al., 1990).

NOD is an inbred strain and should be maintained by brother  $\times$  sister mating. NOD mice have an excitable disposition but breed well. Siblings bred before the development of overt diabetes can usually produce two large litters (9-14 pups each) of which nearly all the pups survive to weaning. Breeders can be protected from developing diabetes by a single injection of complete Freund's adjuvant (Sadelain et al., 1990).

## TRANSGENIC MICE

Since the late 1970s, advances in molecular biology and embryology have enabled scientists to introduce new genetic material experimentally into the germ lines of mice and other animals. The term *transgenic mice*, as used here, means that foreign DNA has been introduced into mice and is transmitted through the germ line. The gene transfer can be performed to introduce new genetic traits or to negate or "knock out" host-gene function by targeted mutagenesis.

Foreign genetic sequences can be introduced into mouse cells, especially in early embryos, by several different methods. The most commonly used method is pronuclear microinjection, in which a solution of purified DNA is injected into either of the two pronuclei visible in a newly fertilized egg (Gordon et al., 1980). Other, less reliable methods include the carrying of the proviral DNA into the cell with a retroviral vector (Jaenisch, 1976) or by electroporation (Toneguzzo et al., 1986) and transformation of totipotent embryonic stem (ES) cells, which are derived from cultured blastocyst-stage embryos (Doetschman et al., 1987). In contrast with microinjection or retroviral insertion, integration of foreign DNA into ES-cell chromosomes can be targeted to specific loci. The specifically modified, undifferentiated ES cells can then be introduced into a recipient embryo in which (it is hoped) they will incorporate into the developing germ line. This approach is used not only for modifying gene expression, but often for introducing targeted mutations by replacement of genes with nonfunctional counterparts, that is, for producing "knockouts" (Mansour et al., 1988).

## Colony Management

Although a transgene causes only a small change in a genome, it can produce dramatic and unpredictable changes that make colony maintenance a challenge. Husbandry and production of transgenic mice have been reviewed (Gordon, 1993) and will be described briefly here.

Colony management can be complicated by several characteristics of transgenic mice, including unpredictable phenotypic effects of transgene expression, pathologic effects of the transgene that compromise viability,