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Raising Laboratory Mice and Rats

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INTRODUCTION

The mouse is probably one of the most widely distributed animals in the world. It has world-wide distribution, having followed man to all of his environments. The mouse probably originated in central Asia, spread to other portions of Asia and to northern Africa, then to central and northern Europe. The mouse came to Mexico and South America on the ships of the southern European explorers, and to the United States and Canada from northern and central Europe with the early colonists.

The mouse was known to the Aryan tribes of Asia 4000 B. C. Pottery depicting mice has been found in Egypt dating back to 2000 B. C. A cult in Asia Minor, northern Africa, Greece, and some of the neighboring islands worshiped the mouse for nearly 3,000 years. Mice were raised in the temples to be used in connection with worship. The mouse was depicted on many ancient coins. The mouse is not native to Japan, but stories of it exist in Japanese folklore. Chinese priests made use of the mouse for many centuries. During the nineteenth century European zoologists bred mice for use in the study of inheritance of color varieties. Their results remained uninterpreted until after the rediscovery of Mendel's laws in 1900.

Production of the albino rat has become a subject of wide interest, principally on account of its many virtues as a laboratory animal. Experimental techniques involving the use of these animals have been employed for many years, but extensive use of the rat began with modern scientific methods of investigation. Experiments with vitamins, hormones, and foods and drugs concerned with animal and human well-being and growth require large numbers of these animals.

The wild gray rat can be tamed and handled, and is sometimes used for special laboratory work. However, various strains of albino and piebald mutants which are more docile and less easily frightened by strange sounds and movements are being produced more extensively for experimental and teaching purposes.

DESCRIPTION

Two species of mice of the family Muridae have been domesticated: *Mus musculus* and *M. bactrianus.* The former is the larger of the two species, has a longer tail, and differs in skull proportions from the latter. The belly is gray. *M. bactrianus* has fewer young per litter and has a white belly. The two species cross readily and the offspring are fertile.

The white rat (*Mus norvegicus albinus*) is a member of the subfamily Murinae of rodents. The genus *Mus,* including rats and mice, contains about 130 species. They are all exclusively of Old World origin. Two species of rats have migrated to the United States in large numbers from Europe. First came the black house rat (*M. rattus*), which has been supplanted largely by the brown or Norway rat (*M. norvegicus*). This latter species now is found

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widely distributed throughout the globe, no doubt largely because it is readily transported by man. Most laboratory rats are probably mutants and domesticated strains of this species. *M. rattus* and *M. norvegicus* are mutually infertile, but available albino strains breed freely with the latter species. Comparisons of hemoglobin crystals and skull shape confirm their relationship.

The albino rat has undergone modifications and the various strains which have been more or less inbred in different laboratories over a period of years show conspicuous differences in size and other characteristics important in animals for laboratory use. The albinos now composing the colonies in various laboratories are not strictly homozygous, even from the standpoint of color, since crosses with pigmented forms give offspring with color markings which could have been derived only from the albino parent.

USE OF MICE AND RATS

Because of its fertility, prolificacy, convenient size, short gestation period, variations, inexpensive maintenance, resistance or susceptibility to certain diseases, and ease of production, the mouse has become a favorite research animal. Its variability has made it a valuable animal for genetic research, and it has been more intensively studied in this connection than any other mammal. It is widely used in medical research, especially in work on cancer. It serves as a medium in which the pathologist cultures disease germs and the physiologist studies various life processes, including the interaction of the hormones involved in reproduction. The embryologist and anatomist have learned much about the early developmental processes of mammals in general from the mouse. Thus the mouse has contributed in wide measure to the biological sciences.

The rat has shared with the mouse its reputation as an animal for research. It has been used more extensively in the field of nutritional investigations than the mouse. It maintains an important place in the study of hormones and in the testing and standardizing of drugs. It also has been a favorite animal for use in animal-psychology experimentation because of its relatively high learning ability as compared with other small laboratory animals.

THE LABORATORY MOUSE

Reproduction and Growth

Ovulation occurs in the mouse at fairly regular intervals of 3 to 4 days, and is not dependent on copulation as it is in the rabbit and some other animals. The female's first estrus occurs at 30 to 40 days of age. The sexes should be separated prior to this. Estrus reoccurs 6 to 24 hours after a female gives birth to a litter and continues for 12 hours or less. Gestation normally lasts 19 to 21 days except in females which are nursing a litter, in which case it may be lengthened from 6 to 16 days. A common practice in the management of a mouse colony is to place three to six females in a cage with a male. After 15 to 18 days the females should be examined for signs of pregnancy, manifested by greatly distended abdomens. Those females which are pregnant should be removed to separate cages, each female in an individual cage. Females usually make a nest in which the young are placed and kept until they are able to run about. The average number of young is 6 to 8, but litters of 12 to 15 are frequent. A mouse at birth weighs about 1 to 1.5 grams, depending on the number in a litter, the young in smaller litters having the greater individual weight. At 4 months of age a mouse weighs about 25 grams, slightly less than an ounce.

At birth the mouse has no hair except the vibrissae, or hairs about the nostrils; no pigment in the skin except in the iris of the eye, visible through closed eyelids; and the ears are thick, bent forward, and attached to the face and cheek. After 2 to 3 days hair appears, at 4 to 6 days the ears detach from the face, and at 8 to 10 days the mouse is fully haired. The incisor teeth erupt at 11 to 13 days and the eyes open at 14 to 16 days. At this time the young

mouse leaves the nest and begins to eat solid food. Young mice may be removed from their dam at 3 weeks of age and the dam rebred. A female is not usually productive after 15 months, but may live much longer. Male mice have been known to live 2½ to 3 years.

Care and Management

HOUSING

The temperature in the building in which mice are housed should be kept at 70° to 80° F. Dampness and drafts should be avoided. There should be good ventilation, however. A variety of types of cages are used, depending on the needs and fancy of the individual. Cages should be of metal, however, to stand sterilization at least monthly by steam or by dipping in a solution of disinfectant. For general purposes the "shoe box" type of cage is satisfactory (fig. 1). The sides and bottom are of sheet metal. The dimensions are 7 by 12 by 5 inches. The cover fits like the cover to a shoe box. A rectangular area in the cover is cut out and the metal replaced with ¼- to ¾-inch mesh hardware cloth. Some absorbent material such as shavings, sawdust, or peanut hulls should be kept in the cages to absorb moisture. Pregnant females should be provided with shredded paper for nesting material. A ½-inch mesh hardware-cloth feed cup is hung over the edge by hooks so that feed is before the mice at all times. This type of feed cup can be used only with the compressed dog biscuit type of food. A glass drinking tube of 6- to 8-millimeter bore extends from an inverted water bottle, into the cage, through a reinforced opening in the top (fig. 1).

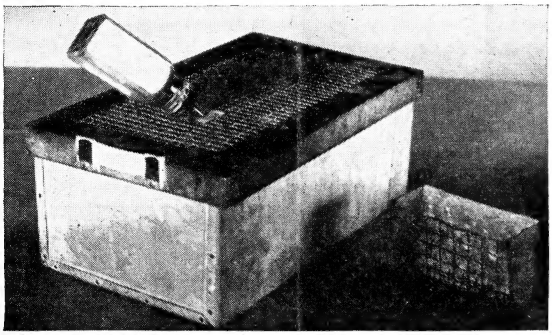

Figure 1.—A metal "shoe box" mouse cage for general use having ½-inch-mesh hardware cloth in the cover. From the inverted water bottle a glass tube extends into the cage. The feed cup is shown at right.

When used to house breeding animals, this cage will accommodate a male and three to five females. This type of cage is also suitable for housing pregnant females and females with their litters to weaning age. As a holding pen for weaned mice, it will accommodate 10 animals.

Another popular type of cage has the same dimensions as the one just described except for the depth, which is 7 inches. The sides are made of ¼- to ⅜-inch mesh hardware cloth. The top of the cage slopes so that the front is about 2 inches lower than the rear. In the top of the cage is a metal door which is slightly smaller than the top. The door is held shut by the weight of the water bottle, which is clipped to it. The water bottle is of the same type as that used with the "shoe-box" type of cage. The glass drinking tube extends from the bottle into the cage through a small circular hole in the metal door. This type of cage has no bottom, but is set in a galvanized iron pan 3½ inches deep, two cages to a pan. Sawdust or shavings are placed in the pan to absorb moisture. When the cages are to be cleaned, the mice are

simply transferred by hand from the one to be cleaned to a clean cage. It is well to have enough cages or pans so that one set may be sterilized with steam or by dipping in a solution of disinfectant while the others are occupied. Both types of cages may be arranged on racks five to six tiers high.

FEEDING

A convenient food for mice is the compressed dog biscuit, which is well balanced as it contains minerals and essential food elements required by mice. A satisfactory mixture also is one composed of 240 parts of rolled oats, 30 of powdered skim milk, 8 of cod liver oil, and 1 of salt.

Various other grains such as ground barley or yellow corn meal may be used in addition to the ones named in the mixture given above. Such foods are fed in a can. Various arrangements may be made for preventing the mice from wasting and contaminating the food.

A piece of carrot, potato, or green leaf of cabbage or kale may be fed occasionally. These should be clean and free from decay.

THE ALBINO LABORATORY RAT

Reproduction and Growth

The rat will reproduce at all seasons, but larger numbers and more vigorous animals are generally obtained in the spring months. Reproduction is lowest through the late fall and winter. The estrous cycle of the rat is 4 to 5 days, and the gestation period approximately 21 days.

The young are born with closed eyes and ears and without hair. Within 21 days they may be weaned and will develop normally when fed a suitable diet such as one of those given in the section on feeding. Although they vary some in the time required to reach sexual maturity, the average animal is mature at 70 to 80 days. It is generally considered good practice to delay the mating of these animals until they are about 4 months of age.

One satisfactory method of mating the animals is to place several females, up to five or six, in a cage with one male, and remove the females to individual cages when they show signs of pregnancy, such as a plug, rapid increase in weight, or sign of erythrocyte, or blood clot. The plug consists of a mass of seminal fluid which has coagulated in the vagina of the female after copulation. It is expelled after a few hours and may be seen in the litter on the floor of the cage. The plug is not always a sure sign of pregnancy, as the fetuses may have died before term, but rapid increase in weight, especially near the end of the gestation period, is usually a good sign of pregnancy. However, if the number of fetuses is small, the increase in weight will be scarcely noticeable. The most certain sign of pregnancy, and that which is routinely used for determining pregnancy in the rat, is the presence of an erythrocyte, or blood clot, in the vagina on the thirteenth day of pregnancy. This may be large enough so that it protrudes and is visible from the outside; or the edges of the vulva may be spread slightly with forceps in order that it may be observed. After the end of the lactation period females should be rested several days, up to about 2 weeks, before remating.

With this method females generally produce four to six litters and then usually are discarded. They are so near to menopause (about 15 months of age) that reproduction is uncertain.

Considerable variation occurs in the number of young to the litter. From 1 to about 10 is the usual number, the average being between 6 and 8. If the large litters are reduced to about 7 at birth the young develop better and the strain of lactation on the female is less.

Other conditions being satisfactory, this procedure should yield about half as many young per week as there are female breeders in the colony.

Sexing of young rats at weaning age and even earlier is often necessary. The most dependable external character by which sex may be determined at birth is the distance from anal opening to genital papilla. This distance is

always greater in a male than in a female of the same age. In animals 21 days of age the developing scrotum and buttonlike appearance of the genital papilla may serve to distinguish the male.

Care and Management

CAGES

The rat will generally gnaw soft materials; consequently metal cages are the most satisfactory for housing these animals. Cages about 20 inches square and 12 inches high with removable screen bottoms and dropping trays are satisfactory for maturing and adult animals (fig. 2, right). However, wire floors are not suitable for young animals from birth up to weaning age.

Pregnant females should be transferred to metal cages which may be somewhat smaller. These cages should contain litter such as wood shavings or

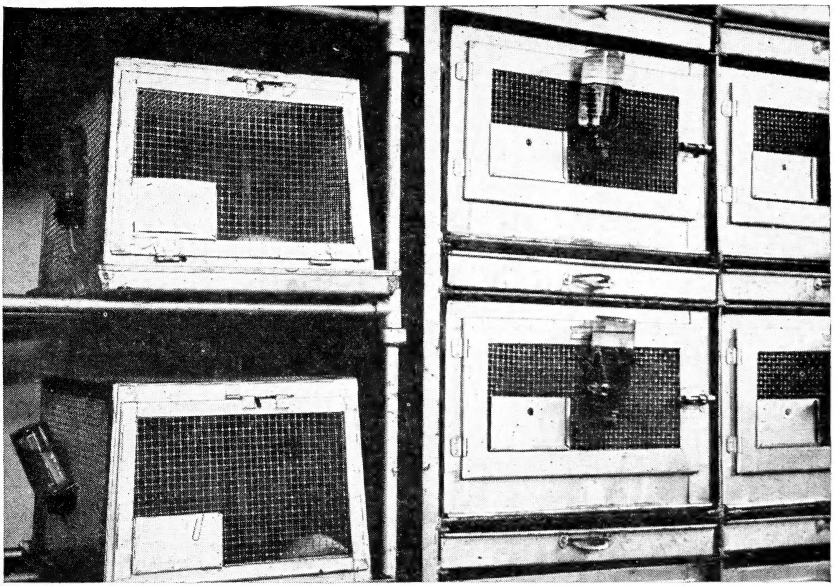

Figure 2.—Two types of cages used for rats. The cages at the left have no bottom, but are set in a pan 1½ to 2 inches deep. This pan is bedded with shavings, sawdust, or other absorbent material. When the pan needs cleaning, the cage is lifted to a pan of fresh bedding material. The cages to the right have wire bottoms, and the droppings fall through to the pan underneath, which contains absorbent material. The pan may be removed for cleaning. The sides and back of this cage are of solid metal. Record cards, held by clips, slide into rectangular holders on the door.

sawdust to absorb moisture and provide nesting material. The practice in some laboratories is to use this type of cage altogether, but the screen-bottom type with tray underneath to catch the droppings is more convenient to clean, as removable paper can be placed in the tray. Breeding rooms should be well ventilated but free from drafts. Temperature should be maintained at 70° to 80° F. and preferably as close to 75° as practicable.

Water bottles and feed cups used for rats are not materially different from those used for mice.

In handling rats, it is well to wear gloves heavy enough that the rats cannot bite through, or to use tongs or forceps. Rat bites may be painful and a source of serious infection unless these precautions are taken.

FEEDING

The rat is capable of thriving on natural foods of considerable variety. Its natural diet is similar to that of human beings, and is derived principally from human-food sources. Table scraps probably were the first diets used for raising these animals. This source of food is variable and undependable for more than a few animals, and is not satisfactory for feeding a rat colony where uniform animals are desired. Nutrition of the rat has been studied probably more thoroughly than that of any other animal; however, doubt exists that it is possible to formulate diets that will produce maximum growth and reproduction in these animals. Numerous combinations of natural foods, however, will produce good results. The purpose for which animals are to be used is generally the chief factor in determining a suitable diet. For example, a standardized animal for use in vitamin assays must be produced on a diet with restricted vitamin content. The vitamins to be assayed must be present in the diet in sufficient quantities for growth, reproduction, and lactation, but must be limited in amount so as not to allow storage of the vitamins in the bodies of the young animals.

A stock ration of this type is generally composed of a mixture of cereal grains such as corn, wheat, and oats, usually in about equal parts, or only one or two of these grains may be used. This grain mixture usually composes from 60 to 80 percent of the diet. The remainder is made up of various vegetable and animal products intended to supplement the protein, mineral, and vitamin content of the diet. Among the ingredients often used are mill concentrates such as wheat germ, alfalfa meal, linseed meal, fish meal, milk powder, liver meal, calcium carbonate, and trace minerals. Table 1 gives examples of diets which, with slight modifications from time to time, have been used successfully for raising albino rats for many generations.

Diet No. 1 is a type that may be fed to produce animals which will be satisfactory for vitamin A and B complex assays. Diet No. 2 probably approaches optimum nutrition more nearly but results in faster growing animals which are unsuitable for vitamin assays.

A less complicated procedure recommended, especially when only a few animals are to be raised, is feeding only the amount the animals will eat of one of the prepared dog feeds produced by commercial feed manufacturers. This may be supplemented with such vegetables as lettuce and carrots.

TABLE 1.—*Suggested diets for laboratory rats*

Ingredient	Diet No.—	
	1 [1]	2
	Percent	*Percent*
Ground yellow corn	36. 00	25. 63
Ground wheat	36. 00	25. 63
Alfalfa leaf meal	3. 00	----------
Linseed meal	10. 00	----------
Fish meal	3. 00	----------
Liver meal	1. 00	----------
Commercial casein	4. 00	----------
Salt	. 50	1. 00
Calcium carbonate	. 50	1. 00
Skim milk powder	4. 00	----------
Yeast (brewers)	2. 00	3. 00
Rolled oats	----------	25. 63
Irradiated yeast	----------	2. 00
Sodium phosphate	----------	1. 00
Potassium iodide	----------	. 01
Manganese sulfate	----------	. 10
Dried beef	----------	10. 00
Dried hog liver	----------	5. 00

[1] With diet 1, fresh whole milk is fed separately 3 times a week.

RECORDS

Some system of marking animals for identification and a book in which to keep the records are often desirable so that the mice and rats in each litter may be distinguished. The type and elaborateness of records will depend upon the purpose for which they are used. For some purposes recording only sire and dam and date of birth of a litter may be sufficient. In other cases more detailed records may be desired. For identification of individual animals, systems of ear notching or toe clipping or a combination of both may be used. The young may be marked when 15 days of age. A punch of the kind used for marking chicks is used for punching the ears. The toes are clipped with small scissors. Usually the bleeding is slight. The bloody areas may be wiped with a piece of cotton saturated with iodine, mercurochrome, or 50-percent alcohol. A numbering system used by the genetics office of the Bureau of Animal Industry is shown in figure 3. By this system, numbers from 1 to 12,999 may be used as a means of identification.

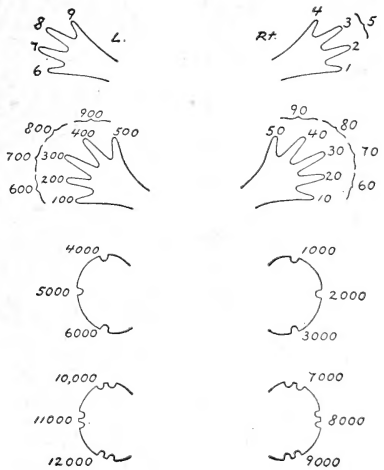

Figure 3.—Diagrams of feet and ears showing a method of numbering by which mice may be identified by toe clipping and ear marking. This method may be used for rats also.

DISEASES

In several instances the mouse and rat are susceptible to the same infections, though in varying degree. In the following discussion of the more common diseases of these rodents, the species affected and degree of susceptibility are given.

Bacterial Diseases

Paratyphoid.—This disease is caused by two species of bacteria, *Salmonella typhimurium* and *S. enteritidis,* singly, together, or with other organisms. Wild mice and rats as well as laboratory stocks of these animals are susceptible. Infection takes place usually through the mouth, by eating food contaminated by carriers of the organism. Carriers within the laboratory colony sometimes serve as a source of infection. Three to six days after infection, mice and rats usually develop conjunctivitis and diarrhea. In animals dying of the disease, the viscera are congested and the liver, spleen, and mesenteric lymph nodes are enlarged. The intensity of these conditions depends largely on the duration of the disease. Mortality may exceed 75

percent. Control of *Salmonella* infection is difficult. Generally it is advisable to destroy the entire lot, and clean and disinfect the cages and premises before obtaining new stock. If the animals are too valuable to sacrifice, they may be divided into small isolated groups and the diseased ones destroyed. Cages should be sterilized. Sick animals that recover may act as carriers and infect the rest of the colony. Surviving males will usually be sterile as the disease causes pathological changes which prevent the descent of the testicles.

Hemorrhagic septicemia.—This disease of mice and rats is caused by *Pasteurella muracida.* Infection takes place mainly by contact with sick animals or carriers which discharge the organism in nasal and conjunctival secretions. Organisms also are air-borne or may be carried in food. The symptoms in acutely affected animals are depression, ruffled coat, loss of appetite, discharge from eyes and nose, and rapid respiration. Death occurs sometimes in a few hours. Infection may cause death even before signs of sickness are noted. At post mortem examination, the viscera are found to be congested and numerous capillary hemorrhages are present on the moist surfaces. In chronic cases, there may be enlargement of the lymph nodes and small, light-colored areas of necrosis in the liver. The mouse is highly susceptible; the rat is more resistant. For control, all affected animals should be removed from the colony and killed. The cages and premises should then be cleaned and disinfected. Highly resistant stocks should be selected for breeding.

Pseudotuberculosis.—A form of this disease, caused by *Pasteurella pseudotuberculosis,* is sometimes epizootic in rats. Infection takes place through the intestinal tract. Another form of pseudotuberculosis which occurs in mice is caused by *Corynebacterium kutscheri* (*murium*). It is a rather infrequent chronic disease manifested by tuberclelike lesions in the lungs; liver; lymph nodes of the cervical region, the mediastinum, and the mesentary; and isolated nodules in the spleen and kidneys.

Mouse septicemia.—This is a relatively infrequent disease of mice caused by *Erysipelothrix muriseptica.* Conjunctivitis, pneumonia, edema of the abdominal tissues, enlargement of the spleen, and small pale gray areas of necrosis in the liver are the chief characteristics of this disease. The organism causing this disease cannot be distinguished from *E. rhusiopathiae* which causes erysipelas in swine.

Pyogenic infections.—Mice and rats may develop abscesses in the subcutaneous tissue of the head, neck, and other parts of the body. Streptococci, staphylococci, and other organisms are causative agents and usually gain entrance through surface wounds. Affected animals should be destroyed and the cages disinfected. *Streptobacillus moniliformis* has been found in the nasopharynx of apparently normal rats but is not responsible for any serious disease problem in these animals. It is highly fatal in mice, however, causing abscesses in the joints and internal organs.

Pneumonia caused by pneumococci and Friedlander's bacilli has proved highly fatal in mice. The disease is rare and usually due to unusual circumstances. *Brucella bronchiseptica* sometimes causes pneumonia in mice and certain pulmonary diseases in rats.

Virus Diseases

Infections of the nervous system.—Encephalomyelitis, a somewhat rare disease, is manifested by paralysis of the hind legs, but not the tail, of mice. It occurs chiefly in young animals.

Meningoencephalomyelitis is also manifested by paralysis of the hind legs.

Choriomeningitis in mice usually occurs as a latent infection without symptoms, although infections resulting in noticeable illness have been reported. Man is also susceptible to the virus.

Infection of the respiratory apparatus.—Mice can be experimentally infected with many types of pneumonia virus, but spontaneous illness caused by these viruses is rare.

Systemic infections.—Infectious ectromelia is an acute disease of mice, and often becomes chronic. In the acute stage polyserositis and acute hepatitis are present. In the chronic stage, edema, vesiculation, ulceration of the skin of the feet, and even sloughing of the feet may result.

The salivary gland disease of rats and mice affects the ducts, salivary glands, and sometimes the kidneys. Inclusion bodies are present in the hypertrophied cells of these organs.

Fungus Diseases

A white ear fungus sometimes grows on the ears of mice. It is difficult to eradicate. If infection is not great the affected portion of the ear may be clipped off. Sodium subsulfate powder will stop bleeding. Tincture of iodine or a 5-percent alcoholic solution of salicylic acid will hasten recovery. Badly infected animals should be killed.

Cancerous Growths

Hereditary tumors, affecting the mammae particularly, are common in certain strains of mice. These occur mostly in aged animals. Strains of animals have been bred for high and for low incidence of various types of tumors. These strains have been used extensively in research on cancer and tumor.

PARASITES

Mice and rats are often parasitized by one or more species of coccidia, but usually these animals are not seriously affected by the organism.

Rats and mice are often parasitized by the larvae of the tapeworm of dogs and cats. These larvae are introduced into the animal colony in bedding or food that is contaminated by the feces of dogs or cats.

Fleas, mites, and lice sometimes infest the mouse or rat colony, but seldom prove fatal. Pyrethrum powder or pulverized tobacco dusted on the animals will usually keep these pests under control. Dipping the animals in a warm, very dilute solution of livestock dip and drying in a warm room are also effective. Cages should be dipped in a solution of cresylic disinfectant.

SELLING AND SHIPPING

Several of the large institutions that regularly use rats and mice now maintain their own animal colonies. However, many institutions depend upon dealers for regular supplies of strains of these animals standardized for special use. There are several producers of standardized strains throughout the country. Names of dealers and of reference books may be obtained on request by writing to the Bureau of Animal Industry, United States Department of Agriculture, Washington 25, D. C. Persons who require large numbers of rats and mice prefer to purchase from well-known dealers or institutions because they are assured of a constant supply of uniform and standard stock.

Many small producers of laboratory animals sell direct to the institutions using them or to dealers who gather up stocks to be sold to the laboratories. Individuals raising these animals should also communicate with the large hospitals and laboratories in their immediate area, as frequently such institutions are desirous of dealing with a dependable producer close at hand.

The Department of Agriculture does not purchase guinea pigs, white mice, or white rats, and has no animals for sale or for free distribution. Persons expecting to make large profits from the raising of laboratory animals should be cautious about expending too much capital, since this, like other enterprises, is subject to disappointments and failures. Most of those who have succeeded have probably done so only after years of patience and experience.

Prices of mice and rats vary, depending upon supply and demand, special purposes for which they were developed, size, age, and various other factors. Mice for laboratory use are usually sold from about 3 weeks of age and upwards and may bring 6 to 25 cents each. Breeding stock is usually sold in

pairs, or in trios of one male and two females. The price requested by breeders also varies. Rats for ordinary use vary in price from 75 cents to $1.50 apiece. Some strains developed for special use may sell for as much as $2.50 to $3 each. A sexually mature rat weighing about 200 grams sells at approximately $1.20. As with mice, breeding stock sells at a higher price.

Mice and rats may be shipped safely for considerable distances if properly crated and if provision is made for food and water. Shipments may be made across the continent by air express, by air freight, or by a combination of either and railway express in about 24 to 30 hours. If the destination can be reached in this time enough feed can be placed in the shipping crates to last the animals until delivery. If a longer period of transit is required, food and water receptacles should be provided in a separate compartment of the crate and arrangements made with the express company for feeding and watering at a stated time. Raw potatoes, carrots, or lettuce leaves should be provided in addition to the dry food. This will make water less necessary for the animals.

A crate 10 by 24 inches and 10 inches deep will accommodate 20 rats. If the trip is for more than 24 hours fewer animals should be put in the crate. A crate 12 by 15 inches and 6 to 8 inches deep is sufficient for 25 to 30 mice. The top of the crate or the top and one side should be of ¼- to ½-inch-mesh wire screen to provide ventilation. Crates should be supplied with shredded paper or wood shavings in which the animals can hide, and in which they can seek protection in cool weather.

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