XXI. LABORATORY RATS

A. INTRODUCTION

1. Origin and Development

The wild brown rat (*Rattus norvegicus*) is thought to have had its original habitat in the temperate areas of what is now the USSR, from the Caspian Sea to Northern China. From this base, it spread over the whole of the Old World during the 18th century, following the movements of modern civilization and to a considerable extent displacing the smaller, less aggressive wild black rat (*Rattus rattus*). The Norway rat did not reach North America until 1775 and there are still some geographic areas, such as the Province of Alberta, that claim to be rat-free. There is no clear reason for members of this species being designated Norway or Norwegian rats, other than the "norvegicus" in its latin name (Harkness and Wagner, 1982; Robinson, 1979).

Captive breeding of the Norway rat probably commenced early in the 19th century, both for fancy and to provide rats to the rat baiting sport (competitive killing of rats by terriers) which has fortunately long been banned. Laboratory rat breeding experiments were first reported from Germany (Circa 1880). Laboratory bred rats were brought to the USA and established first at a Chicago laboratory where they were used for neurological studies. In 1906, some of this stock was transferred to the Wistar Institute in Philadelphia, giving rise to the ubiquitous present-day Wistar strain of rats (Festing, 1979).

The development of today's laboratory rat has been essentially an American initiative and the great majority of the strains of these animals that are presently in use throughout the scientific world have originated in the USA. The history of these developments has been recently reviewed (Lindsay, 1979).

The black rat is occasionally found in laboratory colonies, although rarely used in either biomedical or behavioral research. The cotton rat and the kangaroo rat are both indigenous to North America and belong to the Cricetidae, a separate family from that of the black and Norway rats, which are rodents belonging to the Muridae family.

2. Research Uses

Next to the laboratory mouse, the rat is the most commonly used laboratory mammal, accounting for approximately 20% of the total number of mammals used for scientific purposes (Festing, 1979).

Throughout the past 80 years, rats have been utilized for investigations in almost every aspect of biomedical and behavioral research and testing. Genetic mutants and selection have produced numerous invaluable research models, examples of which will be referred to in the next section. A recent publication dealing with biomedical research applications lists the following areas of biomedical investigation as ones in which the rat is widely used and

particularly useful: toxicology, teratology, experimental oncology, experimental gerontology, cardiovascular research, immunology, dental research immunogenetics and experimental parasitology (Baker, Lindsey and Weisbroth, 1980).

The rat is also the most widely used laboratory mammal in behavioral studies, for which, incidentally, the mouse is not well suited. Rats have traditionally been the animal of choice in much nutritional research, although it should be noted that their natural habit of coprophagy may limit their suitability for certain of these studies.

3. **Selection**

   a. **Genetic Selection**: Rats, like mice are available in various more or less genetically defined varieties, but these are much less numerous and often much less well defined than are the mouse strains.

      The two most common stocks of rats encountered are the so-called Wistar and Sprague-Dawley. Both originated from colonies in the USA and have been disseminated world wide over the past 50 or more years, giving rise to numerous sublines. Each of these sublimes will reflect the differences in selection, environmental influences, genetic sampling and possibly contamination, that have gone into its development. It is important to realize that unless animals of any of the common outbred stocks of rats come from the same subline, they will probably have little in common except their name. For this reason, if genotype is of importance in a particular study, it is advisable to use inbred strains rather than outbred stocks.

      Other common, loosely defined laboratory rat stocks are the Long-Evans, which are hooded animals originating from a cross involving a wild male, and the Osborne-Mendel stock which was established for nutritional studies.

      There are, notwithstanding the above comments, some recognizable gross differences between the common rat stocks, with the Wistar having a wider head and shorter tail than the Sprague-Dawley, while the Long-Evans and other hooded varieties are smaller. Disease susceptibilities and aggressiveness are also said to vary between these stocks (Harkness and Wagner, 1983; Festing, 1979), but in practice these characteristics seem to vary more between selected sublines within the stock than between the stocks themselves.

      A number of less common and more definitely defined stocks and inbred strains have been established by selection or arisen by mutation. These are perpetuated because their genotypes are particularly suited to specific biomedical or behavioral studies. A few of the better known of these are noted here with their primary uses:

ACI:	Congenital urogenital anomalies (Festing, 1979)
CAR; CAS:	Dental caries resistant; susceptible (Navia and Narkates, 1980)
SHR:	Spontaneous hypertensive rat (atherosclerosis, stroke, cardiac drug evaluation, erythrocytosis (NRC U.S., 1976))
AA; ANA:	Alcohol acceptance, non acceptance (McClean, Deitrich and Erwin, 1981)
Brattleboro Rat:	*Diabetes insipidus*; absence of vasopressin (Sokol and Valten, 1982)
BB:	Spontaneously diabetic (Jackson, Buse and Refai, 1984)
Zucker Rat:	Obesity (Deb, Martin and Hershberger, 1976)

b. **Ecological Selection**: Rats are raised in similar ecological classes as have been described in the chapter on Mice. This means that, in addition to conventionally raised animals, germfree, gnotobiotic and barrier-raised rats (with more or less defined microbial burdens) are all available to the investigator. Most commercially obtained laboratory rats today will come under the last named class and will be stated to be specific pathogen-free (SPF).

The term SPF has little relevance unless the specific pathogens are listed, and the latest supplier colony health report is available. In the case of rats for chronic experiments (more than 12 months' duration), the single most important pathogen to be "free of" is undoubtedly *Mycoplasma pulmonis*, which is the principal organism responsible for chronic murine pneumonia (Murine Respiratory Mycoplasmosis or MRM) of rats (Siegmund and Fraser, 1979). MRM has proven in the past to be the greatest single impediment to the successful conduct and interpretation of long-term experiments on conventionally raised rats. MRM is still present, at least as a covert infection, in a number of small institutional rat breeding colonies. Its wide distribution among conventional laboratory rats is the strongest single argument for caesarian derivation, barrier breeding and/or strict colony monitoring.

B. CHARACTERISTICS

1. Development Features

The rat pup at birth weighs about 5 g and is blind, but very active, growing rapidly to 35-50 g by three weeks. The adult male will weigh from 400-500 g with the female being about 100 g less. Size/weight will vary markedly between strains. Adults will continue to increase very gradually in skeletal size throughout life as the rat's long bone epiphyses do not become completely inactive.

Healthy rats will live from two and a half to three years depending on the strain, sex, environmental conditions, and other variables (Baker, Lindsey and Weisbroth, 1980; Suter, Luetkemeier, Zakova *et al.* 1979). Defined flora Sprague-Dawley rodents are relatively short-lived at approximately 2 years for males compared to ACI males that average 31 months (Cameron, Lattuada, Kornreich *et al.* 1982). The albino rat's hair is silky white when young, but becomes progressively coarser and discoloured (yellowish-grey) with age. Rat dentition is typical of that of Muridae with paired, continuously growing, incisors having enamel only on their cutting (front) edges. The three pairs of cheek teeth are present as permanent teeth only (no deciduous dentition); these have open, enamel-free, cusps.

2. Morphophysiology

Multilocular adipose tissue (brown fat) is made up of cells filled with multiple small droplets of brown pigmented lipids that do not coalesce as in the ordinary fat "signet ring" cell. Multilocular fat is diffusely distributed over the dorsal, lateral, and ventral aspects of the neck, as well as retroperitoneally, particularly at the pelvis of the kidney. The prominent accumulation of this tissue in the interscapular region appears gland-like, and has been referred to as the hibernating gland. Its real significance is not yet fully understood, although it is known to be critical to the life of the rat and to play a major role in thermogenesis. The rat, for the above reasons, is a commonly used model for cold adaptation studies (Alexander, 1979).

As in other rodents, the rat's stomach has a large aglandular portion or forestomach, making up over 1/3 of the total gastric mucosa. The glandular stomach has no cardiac glands and is rich in histamine-producing gastric mast cells; pyloric glanus are restricted to the antrium. The large cecum aids in the digestion of cellulose. Atlas guides and reviews on the anatomy, general biological and morphophysiological features of the rat have recently been published (Bivin, Crawford and Brewer, 1979; Olds and Olds, 1979).

The presence of superficial nephrons in the cortex of the rat kidney makes it a good model that is widely used in investigations requiring micropuncture for the evaluation of in vivo tubular function (Windhager, 1968).

A urethral plug has recently been described as a normal feature, present in the proximal urethra of all male Muridae and Cavidae and its absence may be associated with failing health. The plug is chemically similar to the seminal vesicle secretion and to the copulatory plug of the female rodent vagina; its presence does not inhibit urination (Kunstyr, Kupper, Weisser *et al.* 1982).

Hematological and clinical chemistry values for laboratory rats have been reviewed recently in detail, with particular attention to the many variables that may affect these parameters (Ringler and Dabich, 1979).

3. Behavior

Laboratory rats are generally docile and, if handled frequently and gently, will become tame and easily trained. They rarely fight amongst themselves as they live and rear their young communally, often sharing nursing duties.

These behavioral patterns will vary somewhat with the stock and more specifically with the selection that has been practised within a subline.

Laboratory rats, unlike wild ones, are year round breeders. They are omnivorous and will burrow if given the chance.

Rats are intelligent animals that demonstrate a wide range of behavioral traits that are of interest in psychophysiological research (Barnett, 1963). Furthermore, they adapt well to studies in psychobiology and withstand surgery well (Ehrensreund, 1968). Procedures for the stereotactic implantation of electrodes into various centres in the rat brain are well established (Pellegrino *et al.* 1979).

C. PROCUREMENT

1. Sources

In recent years, an increasing proportion of the rats used for research and testing in Canada, the USA, and Great Britain, have been obtained from commercial sources, listings of which are available (ILAR, 1979; CCAC, 1984). A majority of both the commercial and major research institute breeding and supply colonies have in recent years adopted barrier systems. However, small conventional breeding colonies are still numerous and, indeed, are often justifiable on the basis of special research and breeding requirements. Every effort should be made to maintain such colonies in as "clean" a state of health as possible.

Where chronic studies are to be conducted, particularly if the research protocol is stressful, it is unquestionably preferable that barrier raised and maintained animals be used. In Britain, until its recent closure in 1982, the MRC Laboratory Animals Centre has operated a supplier accreditation system which categorized supplier-breeders on the basis of microbial and genetic monitoring. Presumably because it was considered useful by both breeders and users, this service is being continued in a modified form, by the Animal Sciences Association of Great Britain. Although no such scheme exists in either Canada or the USA, most reputable suppliers now offer barrier raised stock from isolator-derived parents and will, on request, provide health reports, genetic data, and other biological information on their rats.

Investigators who are not geneticists may often fail to realize fully the extent of the resource of inbred strains and genetically defined rats available to them. Some useful efforts have been made to put these ever increasing data into perspective so that the choices available may be better appreciated and the animal resources better utilized (Altman and Katz, 1979; Festing, 1979).

The NIH Rodent Repository established in 1975 under the auspices of the Veterinary Resources Branch, NIH, Bethesda, maintains foundation colonies of inbred and congenic strains of both rats and mice, as well as nucleus colonies of a number of outbred and mutant stocks. This resource is periodically catalogued (NIH, 1980) and small numbers may be made available for the establishment of breeding colonies; however, animals are not available from this source for research protocols.

2. **Transportation and Reception**

Transport, particularly by air, is highly stressful to rats. They invariably lose weight, become dehydrated, and should be allowed a recovery period (equilibrium time) of from one to four days, proportional to the time spent in transit. Specific, travel-associated hazards arise from adverse climatic conditions, particularly heat, and from possible exposure to infection.

Direct shipment from supplier to user by properly climatized ground transport is generally well tolerated and is generally practised where feasible. However, in countries such as Canada, where the distances separating supplier and user are often in the thousands of miles, air transport is frequently unavoidable. Problems may be minimized by careful scheduling and proper notification of shipment departure, routing, and ETA. Under these circumstances, proper provision of in transit food and water is of paramount importance (Weisbroth, Paganelli and Salvia, 1977; Van Bekkum, Brouwer, Zurcher *et al.* 1983).

On arrival, a health assessment program should be initiated comparable to that described in the chapter on Mice. Consideration should be given, where physical facilities will permit, to the allocation of a specific room(s) to animals from each supplier, rather than formal quarantine. In any event, the mixing of rats from different sources should be avoided if at all possible. SPF and, more particularly, gnotobiotic animals should be shipped in protective crates and should be quarantined separately behind an appropriate barrier (filter caps, laminar flow rack, separate room, etc.).

3. **Identification and Records**

Cage identification of incoming animals needs to be made immediately on removal from their shipping crate. Identification of individuals will be necessary as they are put on experiment. For short-term trials (two or three weeks) tail colour marking using different positions/colours may suffice. Permanent marking for longer terms will require a system of ear punching or notching (Walker, 1935). Ear tags are not satisfactory, particularly in group housed animals, as they too often get torn out.

Tattoos may be used on the ears of weanling rats or into the palmar and plantar surfaces of the feet in newborn (Baker, Lindsey and Weisbroth, 1980). The importance of proper individual identification and complete record keeping in experimental rodents cannot be over-emphasized and is an essential component of the "Good Laboratory Practices" (GLP) required by government regulatory agencies in the USA, Canada, and numerous other countries.

D. FACILITIES AND MAINTENANCE

1. **Housing**

a. **General Comments**: The discussion and references to facility design in the chapter on Mice are equally applicable to rats. Similarly, the general conceptual comments made in that chapter on the manipulation and

control of the macro-environment (facility and animal room) and the even more critical role of the primary or micro-environment, (cage) need not be repeated here. Discussion of the basic principles involved in the design and proper maintenance of a physical facility for rats has also been provided in some detail in a review of factors affecting biological responses in the rat (Baker, Lindsey and Weisbroth, 1979).

b. **Caging**: The cage provides the primary enclosure in which the rat must live. Its design, fabrication, and contents (water bottles, feeders, bedding, and occupants) will profoundly affect the micro-environment created within it, which in turn may, through variations to the physiology, health, and behavior of its occupants, profoundly influence experimental responses.

   The broad areas of choice in rat caging may be summarized as follows:

   i. Shoebox vs wire mesh floor.

   ii. Bedding vs paper-covered dropping trays.

   iii. Metal vs plastic.

   iv. Punched metal vs welded rod vs wire mesh tops.

   v. Filter top vs no protective tops.

      There are pros and cons for each of these options which have been subject to review in several publications and symposia on animal care (Baker, Lindsey and Weisbroth, 1979; Clough, 1976; Woods, 1980; Lane-Petter, 1976). Obviously the choices made will depend on such things as experimental objectives and equipment available; however, there are a few points that should be kept in mind when giving consideration to these alternatives:

   vi. Galvanized metal should be avoided in long-term toxicity and nutritional studies, as rats will ingest zinc from these.

   vii. No metal whatsoever should be used in the cages or ancillary equipment in contact with the animals in trace element studies (chromium, nickel, etc.). Metal free individual suspended cages of simple plastic construction, adapted to standard racks, have been described (Polansky and Anderson, 1979).

   viii. Single housing of rats will incur marked changes in their disposition, adrenals, thyroids, microsomal liver enzymes and such behavioral parameters as alcohol consumption (Baker, Lindsey and Weisbroth, 1979).

   ix. Wire floor inserts may be useful in polycarbonate (clear) or polypropylene (translucent) shoe box cages in carcinogen bioassay or nutritional studies. Ammonia buildup is significantly reduced if

absorbent bedding is used under the insert, and will be minimal if direct contact bedding (no insert) is used. However, the latter situation enhances aerosol spread contamination significantly (Raynor, Steinhagen and Hamm, 1983).

x. Any advantages derived from the use of filter caps must be carefully weighed against the changes these induce in the cage environment, particularly in ammonia buildup. As was recommended for mice, if it is decided that filter caps are to be used, it is essential to increase frequency of cage cleaning, decrease cage population, reduce room temperature, and maximize air changes.

2. **Environment**

a. **Bedding/Ammonia**: Comments on the influence of cage design on ammonia buildup have already been made. There is ample evidence to implicate ammonia in exacerbating respiratory problems, particularly from mycoplasma infections in the rat (Gamble and Clough, 1976; Broderson, Lindsey and Crawford, 1976). There is, however, also evidence that environmental ammonia, at concentrations commonly found in animal holding rooms (below 100 ppm), probably causes minimal adverse effects on rats that are healthy to start with (Schaerdel, White, Lang *et al.* 1983).

Criteria for bedding and the effects of different types of bedding were briefly discussed in the chapter on Mice. The quality control and care in storage of these materials are of special importance, particularly as natural products such as these, having a such long shelf life, often become contaminated by wild rodents and cats. Contaminated bedding may introduce disease (particularly mites and tapeworms) into the colony. Bedding for the barrier colony must be sterilized. Unfortunately, there is at least a slight possibility that both ethylene oxide and steam sterilization may introduce their own contamination to some bedding materials (Kraft, 1980; Wirth, 1983).

b. **Temperature and Humidity**: These environmental parameters were discussed in the chapter on Mice and have been reviewed in some detail in several publications (Baker, Lindsey and Weisbroth, 1979; Clough, 1976; Woods, 1980; Weihe, 1976). The temperature and humidity ranges for rats, suggested in Volume 1 of this Guide (CCAC, 1980) are 20^{o}-25^{o}C (68^{o}-77^{o}F) and 50-55% respectively. Rats can, particularly if given a reasonable period of acclimatization, adapt with apparent comfort to far wider temperature ranges. This is especially true of the cold end of the temperature scale, where they will readily adjust to constant temperatures of 10^{o}C (50^{o}F) or less. Humidity ranges of from 40-70% are also tolerated without apparent adverse effects (Weihe, 1971; NRC U.S., 1977). However, temperature and humidity should be kept relatively constant throughout a given experiment to minimize the considerable indirect effects of fluctuations on research data, through altered food and water consumption (Weihe, 1971) and increased susceptibility to certain diseases (Flynn, 1967). Body weight gains do not differ among rats raised within the temperature range of 18^{o}-28^{o}C (64^{o}-82^{o}F), but are reduced beyond these extremes. Food intake does not differ at temperatures between 20^{o}-26^{o}C (66^{o}-79^{o}F), nor does water intake between 12^{o}-26^{o}C

(54°-79°F). Hematological and serum biochemical values are constant between 20°-26°C (Yamauchi, Fujita, Obara *et al.* 1981).

c. **Air Exchange**: Ventilation is of particular importance in the rat room due to the high level of susceptibility of this species to respiratory diseases. Recommended air exchange rates for 100% fresh air vary from 10-20 changes per hour depending on the population density and whether or not filter caps are in use (CCAC, 1980).

Undirectional air flow systems and filter racks have been recommended to permit safe recirculation of air through the removal of particulate matter that might act as fomites for toxic and pathogenic substances. These systems involved HEPA filters and at least 10% fresh air exchange (Baker, Lindsey and Weisbroth, 1979). Laminar flow racks have been used successfully as mini-barrier areas within conventional animal rooms. The use of relatively inexpensive portable filter units should be considered for the reduction of animal odours, ammonia, and levels of airborne micro-organisms. These may prove particularly useful in rooms not originally constructed for animal housing and not adequately modified for that purpose (Baskerville and Seamer, 1982).

Viral cross-contamination between racks is not necessarily eliminated in mass air flow (laminar flow) enclosures, and rapid cage to cage bacterial cross-transmission may also occur in such systems. Consequently, it is advisable that rats housed in such enclosures should have been acquired from the same source, thus having comparable microbial profiles (Thigpen and Ross, 1983). The above points underscore the importance of establishing an adequate monitoring regime if a barrier system is to prove effective and reliable (see chapter on Mice).

d. **Light and Noise**: The significance and influence of light on rats is comparable to that described for Mice. Retinal damage associated with light exposure and age in various albino stocks and strains is generally comparable to that in albino mice (Anver and Cohen, 1979).

Rats have an acute sense of hearing, with sounds of 160 decibels causing mechanical injury of their ears, as they do in man. Consequently, animal room noise should be maintained at below 85 decibels (Baker, Lindsey and Weisbroth, 1979). Noise at 107-112 decibels for 1 1/2 hours daily for five consecutive days has been associated with significantly enlarged adrenals, a relative eosinopenia, leukocytosis and increased food intake with a lesser rate of gain than in controls (Nayfield and Besch, 1981).

E. NUTRITION

1. Nutrient Requirements

Rat ration nutrients are generally based on the recommendations of the National Research Council (U.S.) Committee on Nutrient Requirements of Domestic Animals (NRC U.S., 1978). Much of the experimental data on these requirements, particularly in so far as the effects of micro-nutrients (vitamins, minerals, etc.) on physiological and pathological processes are concerned,

have been based on studies on laboratory rats. As a consequence, firm conclusions may be made on the requirements for most of these nutrients (Rogers, 1979). However, there is less assurance based on direct evidence as to the rat's requirements for some of the major nutrients, particularly in terms of energy-protein interrelationships. Many of the values used in ration formulation are based on information obtained from monogastric farm animals such as pigs (Ford and Ward, 1983).

The dietary content of nutrients for rats has been the subject of a recent, thorough review (Rogers, 1979) which concludes that; a) an adequate protein level for the support of growth, gestation, and lactation is probably 12%, with adult, non-pregnant, and aging rats requiring lesser protein and amino acid content: b) the minimum fat content in the ration must be not less than 5% although rations often contain up to 15%; c) Linoleic acid should make up at least 0.3% of the diet by weight. This essential fatty acid (EFA) is readily available and may be converted by the rat to arachidonic acid which is the major EFA in cell membranes, and is an important precursor of prostaglandin (Rogers, 1979).

The composition of the diet is a very important experimental variable that often tends not to be adequately controlled and is rarely recorded (except in nutritional experiments) adequately enough to permit its proper evaluation as a variable or for a precise repetition of the original protocol (NAS, 1978).

The rat's natural habit of extensive coprophagy may considerably distort and obscure the influence of diet on experimental results. Possibly as much as 50-65% of the fecal output of rats on adequate diets may be reingested by coprophagy (Neale, 1982); presumably this trait would be increased by deficient diets. Use of wire-mesh floors does not prohibit coprophagy and rats will go to great lengths to gain access to their feces (Waynforth, 1980).

Nutrient requirements sometimes need to be modified extensively to meet the operant requirements of various genetic models (Hess, Newsome, Knapka *et al.* 1981) or to establish nutritional models by creating controlled deficiencies. The latter are widely used in carcinogenesis and toxicity studies (Rogers and Newberne, 1975; Carroll, 1975).

2. **Feed and Water Supply**

A majority of laboratory rats are maintained on commercial dry pelleted feeds. These will, in most instances, prove satisfactory provided they are obtained from a reputable manufacturer, are reasonably fresh, and properly stored. These factors as well as the concepts implicit in the different types of formulation, and in the spread of contaminants through the feed, were discussed for rodent diets in general in the chapter on Mice.

Adult rats will eat from 12-30 gms of dry food pellets daily and, if the diet is complete, do not require any supplementary foods.

Defined diets, either semi-synthetic or chemically defined, often need to be used in studies on carcinogenesis and in toxic substance bioassays, as well as in nutritional experiments. When such diets are needed, there are many

advantages to their being prepared and fed in an agar-gel base. Use of this type of base tends to reduce body weight and increase longevity, give optimal growth, minimize wastage and greatly reduce the risk of animal cross-contamination and of personnel exposure (Clapp and Bradbrook, 1982; Sansone and Fox, 1977). Rats drink 140 ml/kg body weight daily of water. They will, on the average drink 2 ml water for every gram of dry food consumed, but much less with gelled feeds which will contain approximately 50% water. Considerations for the acidification of the water supply, particularly in automated systems, are the same as for mice.

F. REPRODUCTION

1. Maturation and Sexing

Puberty occurs at 50-60 days in both sexes, with the vagina usually opening about two weeks later. The testes descend well prior to puberty, usually at about weaning age. The rat testes are retractable.

Rats breed year round and do not exhibit appreciable seasonality; however, litter frequency will decrease in the winter months unless artificial light is used to maintain approximately 14 hours of light.

Litters are weaned at three weeks, by which time pups will weigh from 40-50 g. Breeding may occur at any time after the vagina is open, but should be delayed until the female is at least 90 days old and approximately 200-275 g, depending on strain. Females will continue to raise litters into old age, although they become progressively less regular after 12-15 months. Productivity, (size of litters, numbers weaned, etc.) will usually start to fall off before the female is quite a year old.

Young males should not be used as sires until at least three months or until they weigh 275-350 g. Sexing of pups can easily be done on neonates by comparing the ano-genital distances between litter mates. This distance in the male will be twice that in the female. In addition, the male genital papillae are larger. Nipples in the female pups are visible by about one week and the testes in the male can usually be observed in the scrotum by three weeks if the pup is held with its head up.

Weanling rats should be segregated by sex by about seven weeks to avoid precocious breeding.

2. Estrous Cycle

Rats are polyestrous, with acceptance of the male and ovulation occurring every fourth or fifth day through a 12-14 hour period. The stages of the cycle are easily identified cytologically from vaginal smears (Young, Boling and Blandau, 1941). Due to its shortness and regularity, the rat's estrous cycle will rarely need to be monitored cytologically except where timed pregnancies are required. Successful mating, may be confirmed by observing the copulatory (vaginal) plug in the female (this is more readily observed in the mouse), or by identifying spermatozoa in the vaginal smear (present for at least 12 hours).

Timed pregnancies are usually accomplished by overnight pairing. Greater precision, with essentially the same conception rate, will result from a two hour pairing on the morning that an estrous vaginal smear is observed (Bertholet, 1981).

Estrus may be synchronized and its onset stimulated by introducing a male into a cage of females. This so-called "Whitten effect" is a response to male pheromones (odours), and is much more pronounced in mice than it is in rats (Harkness and Wagner, 1983).

Pregnancy lasts for 21-23 days. However, the time from fertilization to birth may be lengthened to 30 or more days due to delayed implantation following post- partum breeding. This delay tends to be proportional to the number of young being suckled by the female. Post-partum estrus occurs within 48 hours of giving birth and matings at that time are better than 50% successful. Failure to conceive at that time will delay breeding until two to four days after the litter is weaned.

3. **Breeding Systems**

   a. Monogamous mating necessitates the maintenance of very many males and cages, but facilitates record keeping and post-partum breeding. Consequently, this system is often favoured in small colonies of inbred animals.

   b. Polygamous matings (harems) are more commonly used and may involve from two to six females being caged with one male. When several females are in a harem, it is usual to remove pregnant animals prior to parturition and return them as soon as their young are weaned. In this way, interference with the young by the male, losses from excessive crowding, and the mixing of litters are avoided. However, *post-partum* breeding does not occur. Harems employing only two females can be left together without any appreciable-fear of the above disadvantages, particularly if the young are removed for up to 12 hours the day after birth to avoid problems associated with post-partum breeding.

   c. Other breeding systems sometimes used include:

      i. Rotating a male between seven separately caged females, allowing one week with each immediately following weaning.

      ii. Cross-fostering pups to establish unisexual litters of up to 14 pups each per female. This will not cause rejection if done properly, but requires either a very large colony or a synchronized breeding system so that a number of litters will be born at one time (Lane-Petter and Lane-Petter, 1971). Such a system has obvious advantages for production and sex manipulation, although there is some indication that the unisexually raised females themselves may produce smaller litters (Sharpe, Morris and Wyatt, 1973).

4. **Factors Affecting Fertility and Reproduction**

a. **Genetic**: A great deal of variance in reproductive performance has been recorded between different stocks, sublines and strains of laboratory rats. Obviously much of this variance will be a reflection of multifactorial differences in genotype that have occurred, more or less accidentally during selection for other characteristics. An example of this would be the reported increased fertility of aged ACI male rats over that of Sprague-Dawley males of similar age; a feature that correlates with a six month longer life expectancy for the former strain (Cameron, Lattuada and Kornreich, 1982).

Other examples of gene mediated differences in fertility can be related to the effects of a single gene, as in the significant reduction in the litter sizes of jaundiced (j/j) Gunn rats, compared to those from (+/j) non-jaundiced females of the same strain (Davis and Yeary, 1979). Mutations, when they occur spontaneously in a rat breeding colony, will invariably be more or less detrimental. Deleterious mutations are frequently expressed in impaired reproductive performance and should, as a general rule, be bred out by negative selection (Lane-Petter, 1972).

b. **Environmental**: The recommended temperatures and humidity levels for rat colonies have been referred to above. Probably more critical is the avoidance of excessive fluctuations, which ideally should be held to less than 1 C. In practice, it seems that none of the reproductive parameters of the rat are affected through a wide range of constant temperatures at 2 C intervals from 12^{o}-28^{o}C (Yamauchi, Fujita, Obara *et al.* 1981).

Cage type, floor area, type of bedding material, crowding and frequency of cleaning are all factors that have been considered to exert varying degrees of influence on breeding performance. The role of these factors and that of nutrition and light, which are somewhat more clearly defined, have been reviewed by several authorities (Baker, 1979; Lane-Petter and Pearson, 1971; Farris and Griffith, 1963).

G. RESTRAINT AND MANIPULATIONS

1. Handling and Physical Restraint

The repeated handling of rats that occurs during many experiments may present a major uncontrolled variable if routine, non-stressful procedures are not followed.

The use of forceps and gloves to pick up and handle laboratory rats is rarely justifiable and will invariably be resented by the animal which will tend to struggle, become hurt, and consequently less amenable to future handlings.

Rats will be gentled by the warmth of a bare hand, rapidly ceasing to struggle, and becoming progressively more tractable at subsequent handlings. Methods for grasping and picking up rats have frequently been described and illustrated (Harkness and Wagner, 1983; Kraus, 1980; Green, 1979). In general the procedure involves holding the animal at the base of its

tail with one hand while grasping it with the other over its back and ribs so that the thumb and a finger are behind the elbows, pushing them forward; the first finger may be place under the neck behind the mandible. When grasped properly, the rat cannot bite even if it should try. Several specific points should be kept clearly in mind when first undertaking to pick up a mature laboratory rat:

a. Do not grab or make sudden hand movements, let the rat sniff your hand since it sees poorly and needs to be able to sniff out the lay of the hand.

b. Do not be afraid; nervousness is contagious.

c. Do not squeeze over thorax or around throat, it will impede breathing and make the rat struggle.

d. Do not pick a large rat up by the tip of its tail, the skin may easily break if it struggles and may actually be pulled off from the underlying tissues of the tail.

e. Pick up smaller rats by the base of the tail—never further back, as they may swivel around, climb up their own tail and bite you, because they become frightened.

f. Try to make sure that all the animals in an experimental group of rats are handled by their attendants as often as possible prior to commencement of an experiment.

Several types of mechanical restraining devices are on the market. These have been designed for a variety of purposes such as the injection and withdrawal of blood, short-term cannula collection of body fluids, and restraint (positioning) during surgery. Some of these have been described in some detail by Kraus (1980) who, in addition, gives a number of useful references to the construction of economical homemade restraining devices.

2. Sampling and Dosing

An extremely useful and comprehensive compendium of devices and techniques applicable to all the main areas of research in which rats are used has recently been published (Petty, 1982). This monograph and the previously mentioned chapter by Kraus (1980) constitute very useful sources of information on methodologies in sampling, dosing, and monitoring body functions in experimental rats.

a. **General**: The variance that even the most simple and most common stressors, such as moving the rat cage or an altered sampling technique can effect, is far greater than is generally appreciated. A study of stress-linked blood and circulatory characteristics has shown the extreme difficulty that exists in obtaining undisturbed values. In that study, the stress from moving the rat cage from its shelf was sufficient to alter a majority of the 25 parameters measured by increments of from 10-500% over those of controls through 2 to 5 minutes following the first touching

of the cage (Gartner, Buttner, Dohler *et al.* 1980). Other studies have shown that the sequential removal of 1 ml blood every second week, which is a generally accepted level, while not disturbing most hematological values did cause a persistent decrease in weight gains (Cardy and Warner, 1979). Such variables may not be amenable to total elimination, but it is important that they be recognized and every effort should be made to minimize their effects.

b. **Blood Sampling**: Numerous procedures have been described and reviewed (Waynforth, 1980; Kraus, 1980; Petty, 1982). The choice of a procedure will usually depend on the volume required and the frequency of sampling. Other considerations must include the possible effects of anesthetics and of the technique employed, on the blood constituents of concern.

   For small-volume repeated sampling, bleeding from the orbital sinus is widely advocated (Harkness and Wagner, 1983; Waynforth, 1980; Kraus, 1980; Petty, 1982). This is normally done with anesthesia; healing is rapid and complete and the procedure may be repeated in a few days. The commonly used technique for orbital sinus bleeding in the rat is the same as that briefly described in the chapter on Mice. However, greater quantities of blood (up to 4-6 ml from 115-130 g rats) may be collected by this procedure if a larger pipette (13 x 100 mm) or multiple small heparinized commercial tubes are used (Lane-Petter and Pearson, 1971). When attempting orbital sinus bleeding for the first time, it is essential to use an anesthetized rat and to have refreshed one's memory of the anatomy of the region (Timm, 1979). It should be noted that the procedure, if used repeatedly, will cause local tissue damage which involves the harderian gland. The changes induced should be differentiated from those due to sialodacryoadenitis virus infection (SDA) (McGee and Maronplot, 1979).

   Cardiac puncture is commonly used in rats for the rapid collection of fairly large samples of 5 ml or more of blood. This procedure must only be undertaken on anesthetized rats. A 24 gauge 1/2 in needle may be used to penetrate the thoracic wall. Entry should be at a 45 angle between the 5th and 6th ribs to the left of the sternum. If the left ventricle is penetrated, blood will rapidly flow into the syringe.

   Modifications of the cardiac puncture technique to adapt it to smaller rats and neonates, as well as a variety of venipuncture procedures have been fully reviewed recently (Waynforth, 1980; Kraus, 1980; Petty, 1982).

c. **Catheterization**: The tail blood vessels are often chosen as sites for relatively short-term, indwelling cannulae. It the rat is held in an appropriate restraining device, it need not necessarily be maintained under anesthesia throughout the period of catheterization. Either the lateral tail vein or the caudal artery may be used. Venipuncture of these vessels in the rat is more difficult than in the mouse due to the thickness and hardness of the skin. Methods for softening the skin and dilating the vein are similar to those recommended for mice. If the procedure is to be terminal, the vessels may be exteriorized. Indwelling catheters of

polyethylene tubing may be protected by a wire mesh sheath taped to the tail for continuous infusion over several days (Waynforth, 1980; Rhodes and Patterson, 1979).

Chronic indwelling catheters are commonly implanted into the jugular vein, primarily for pharmacokinetic studies (Waynforth, 1980; Petty, 1982). Cannulation of the cranial mesenteric vein has been described for delivery of materials (pancreatic Islet cells, for example), into the portal system over a prolonged period (Zammit, Toledo-Pereyra, Malcolm *et al.* 1979).

d. **Sampling**:

i. Urine—Use of metabolism cages is the easiest way to collect both urine and fecal samples. Commercial metabolism cages are designed to separate urine and feces without allowing access to other contaminants such as food, water, and hair. These cages may be either of metal or plastic material. Several laboratory fabricated and modified metabolism cage designs, as well as other methods of collecting urine and feces, have been described (Waynforth, 1980; Kraus, 1980; Petty, 1982).

Urine can be collected from rats in hanging wire bottom cages by using crumpled aluminum foil attached under the wire mesh of the cages—1 ml or more of urine being collected by this method from most rats within an hour (Black and Claxton, 1979).

Bladder centesis, urinary fistula, catheterization in females and external drainage catheters in males have been described. Most of these procedures should only be invoked where a total collection is essential or in terminal experiments.

ii. Feces—Collection of feces without contamination may be done by using anal cups, which will be more effective on male rats as their ano-genital papillary (urethral) distance is twice that of the female. The construction and use of fecal cups has been described (Smyth, 1979).

iii. Other Body Fluids—Methods for sampling bile, semen, pancreatic juices and various other substances may be found in either the review by Kraus (1980) or the monographs by Waynforth (1980) and Petty (1982).

e. **Oral Dosage and Forced Feeding**: These procedures are easily performed on the well-handled (tame) laboratory rat. Usually no gag will be necessary for the passage of even a #8 French catheter, although use of a simple gag is described and should be available (Waynforth, 1980). Fifteen cm is the approximate distance from incisors to pyloric stomach in adult rats and the stomach tube should be marked at that point; it should also be lubricated prior to passage. For most purposes of occasional dosage, the use of a ball tipped, curved, gastric inoculation needle will prove the method of choice. The rat should be restrained with the index

finger and thumb on either side of the neck behind the mandible. The ball tip of the needle is rotated through the diastema, between 1st molars and incisors, and passed back over the tongue to the pharynx and, as the rat swallows, gently on into the esophagus (Kraus, 1980). Alternative procedures of handling and passage have also been described and illustrated (Waynforth, 1980; Petty, 1982).

H. ANESTHESIA

1. General Procedures

Much of the risk associated with anesthesia in the rat can be attributed to the effects of MRM. The risk factor from this condition is particularly pronounced when prolonged anesthetic maintenance with an inhalant agent is necessary (Waynforth, 1980).

Atropine should be given i.m., at 0.05 mg/kg about 30 minutes in advance of induction to reduce salivation, particularly if ketamine or pentobarbital are to be used.

Hypothermia is always a risk in anesthesia of small rodents, due to their high metabolic rates, and should be guarded against, both during surgery and the recovery period, by the judicious use of heat lamps, thermal pads and/or hot water bottles.

Assessment of the depth of anesthesia is particularly critical because of the rapidity with which cardiac and respiratory failure can follow the first signs of apnea and lead to death in the rat. The foot pad and ear pinch reflexes are the most sensitive indicators of anesthetic depth and one should closely observe the animal for signs of impending apnea such as mucous membrane cyanosis and alterations in the pattern of respiration. The anesthetist should be prepared at all times to administer supplementary O2 and to initiate artificial respiration.

2. Injectable Anesthetics

Neuroleptanalgesics have many advantages as injectable anesthetics for rats, even though barbiturates (usually sodium pentobarbital) are still the most commonly used agents for this purpose (Kraus, 1980). This is surprising in view of the narrow safety margin with the latter agent, in which the anesthetic dose is about 40 mg/kg and the LD-50 is only 60 mg/kg (Harkness and Wagner, 1983). A risk comparison study between etorphine-acepromazine neuroleptanalgesia and pentobarbital sodium anesthesia has shown significantly fewer deaths from the former (Fisker, Stage and Philipsen, 1982). Individual variations to pentobarbitone are very marked, with the risk associated with MRM being very high and post-operative recovery time often excessively prolonged. A further advantage to neuroleptanalgesics is that the effects of their narcotic element can be rapidly reversed by specific antagonists (Waynforth, 1980).

Fentanyl-droperidol (Innovar-vet) has proven a useful agent in the rat and, at 0.2-0.4 ml/kg, i.m. produces an adequate anesthetic state for superficial

surgery. For intra-abdominal procedures, fentanyl-droperidol at 0.2 mg/kg, i.m. combined with diazepam 2.5 mg/kg i.p., as a muscle relaxant, gives good results for periods of up to half an hour. For longer lasting anesthesia, 0.3 mg fentanyl fluanisone may be substituted for the fentanyl-droperidol (Harkness and Wagner, 1983; Waynforth, 19780; Kraus, 1980).

Ketamine hydrochloride i.m. is unpredictable in rats and produces poor muscle relaxation. However, a combination of 90 mg ketamine with 5-8 mg xylazine per kg i.m. is an effective and safe anesthetic for pregnant rats (Stickrod, 1979).

3. **Inhalant Anesthetics**

These agents have the advantage of greater ease of control over depth and duration of anesthesia, as well as usually being followed by a relatively smooth, rapid recovery.

The semi-open drop (Bell jar) method of induction is still the most common means of induction with volatile anesthetic agents, followed by open-drop nose-cone maintenance. It is also probably true that ether is the most widely used agent despite its disadvantages of inducing excessive salivation and irritation to the respiratory epithelium. The continued popularity of ether may probably be attributed to tradition, low cost and ease of use with minimal equipment. Certainly, it is easy to control, relatively safe and gives good muscle relaxation for procedures lasting up to an hour, being followed by rapid recovery (Waynforth, 1980; Green, 1979). Its use has obvious appeal and advantages for the many investigators for whom surgery is only an occasional requirement and who are not themselves primarily either surgeons or anesthetists. The high inflammability and explosive nature of ether must always be kept in mind and proper procedures for its storage, once the can has been opened, should be strictly adhered to.

Where surgery is a major component of experimental protocols, a more sophisticated approach to inhalation anesthesia is indicated. For this, methoxyflurane is often the agent of choice as it is safe and may be used at 0.5-1.0% with O2 to produce a prolonged and stable anesthetic state.

Anesthetic machines are available commercially for use on small rodents or along with vaporizers and other such equipment, may be improvised (Kraus, 1980; Green, 1979; Norris and Miles, 1982; Cooke, 1976). Induction of anesthesia with methoxyflurane using the semi-open, Bell jar method as for ether, will prove both slow and very expensive and is not recommended. However, if a pre-anesthetic injection of ketamine or a tranquillizer is used, methoxyflurane may be used effectively when given by vaporizer or improvised mask (Waynforth, 1980; Levy, Zwies and Duffy, 1980).

Halothane is difficult to administer to rats and is not generally used although it has been successfully applied to rats and mice using an improvised closed anesthetic unit to control anesthetic levels, remove carbon dioxide and contain anesthetic gases (Mulder and Hauser, 1984).

Endotracheal intubation may be advisable if surgery is to be very prolonged and where controlled mechanical ventilation may be necessary (thoracic and some abdominal procedures). However, as intubation is difficult in the rat, due to the anatomy of the rodent oropharynx (Olds and Olds, 1979), persistence, practice, and care will be needed if any of the several published procedures are to be successfully accomplished (Petty, 1982; Thet, 1982; Alpert, Goldstein and Triner, 1982).

I. EUTHANASIA

Acceptable procedures and agents for the killing of laboratory rats have been listed in Volume 1 of this Guide with comments on the underlying principles in humane killing (CCAC, 1980).

Selection of the procedure/agent to be used will necessarily be influenced by the requirements of the particular experimental protocol concerned. The main conditions (limitations) in this regard are the possible affects on physiological/biochemical parameters to be measured and on intended special uses of tissues (e.g., effect of barbiturates on hepatic microsomal enzymes for use in cell cultures). Some of these problems were referred to above under sampling (bleeding) and/or in the chapter on Mice. Recent reviews on the physiological effects of various means of euthanasia have concluded that CO_2 was among the least disruptive agents available for general use. Euthanasia with this gas is quick, apparently painless, and quite acceptable provided the container (chamber) is prefilled with CO_2 (Kraus, 1980; Feldman and Gupta, 1976).

J. HEALTH CARE AND DISEASES

1. General

During the first half of this century, laboratory bred rats were frequently subject to outbreaks of clinical diseases of bacterial and parasitic origin. Through selection, improved husbandry, and effective health assessment programs, the nature of the disease problem in laboratory rats has changed markedly over the past 30 years. Overt bacterial disease and major colony health problems from parasites are rare in today's rat colony. The threat now is from sub-clinical infections mostly involving viruses and mycoplasma. The challenges are in their detection and elimination.

Not the least important aspect of health care of laboratory rodents is that of the assessment of the impact of latent infections on the increasingly precise and demanding measurements required of biochemical data. Presumably shifts in the disease spectrum of laboratory rats will continue to occur in response to altering genetic and environmental requirements in biomedical and behavioral research. The role of laboratory animal medicine would, therefore, seem increasingly to involve the development of a better understanding not only of the role of microorganisms in such shifts, but also that of environmental and genetic factors.

At present, the state-of-the-art of health care for laboratory rats relies heavily on the use and establishment of various levels of barrier maintenance for

"clean" rats that are the SPF progeny of isolator derived parents. The problems are ones of the effectiveness of various barriers and of weighing need against cost in light of the research objectives. Clearly, the effectiveness of the barrier will be directly reflected in the efficacy of disease control, even though health care in toto obviously involves many other controllable factors (e.g., nutrition, husbandry, etc.) which will be effective under conventional as well as barrier conditions.

Several comprehensive overview chapters on various aspects of health care and diseases in rats may be found in The Laboratory Rat, Volume I, Biology and Diseases (Lindsay, 1979). Other recent monographs on diseases of laboratory rodents (Harkness and Wagner, 1983; Russell, Johnson and Stunkard, 1981) should also be referred to, to supplement the brief annotated listing of rat diseases given here. Treatments have not been included for infectious diseases as they are rarely justifiable in a colony. Either elimination followed by prevention or the use of barrier conditions are the only feasible approaches to the control of contagion.

2. **Infectious Diseases**

a. **Mycoplasmosis**: Murine respiratory mycoplasmosis (MRM) has been advocated as a more appropriate and specific name for CRD (Cassell, Lindsey, Baker *et al.* 1979). *Mycoplasma pulmonis* is the organism that plays the major role in chronic respiratory infections in the rat although other bacterial and viral agents may sometimes—be involved. The syndrome is expressed by various signs which may develop either separately or in combinations. Some of these signs have been thought of and described as separate disease entities as in the cases of:

i. Otitis media and/or interna which induces a characteristic circling when the rat is lifted by its tail.

ii. Rhinitis with sneezing and a blood flecked discharge around the nostrils.

iii. Pneumonia with laboured breathing and progressive debility.

M. pulmonis may also infect the genital tract, particularly of females. When present in this form in a breeding colony it may prove a major cause of low fertility through reduced litter sizes or even complete infertility (Cassell and Hill, 1979).

Fortunately, MRM has, over the past few years, been largely irradicated, at least from most major breeding colonies. However, this should not lead to complacency as *M. pulmonis* is still around in a number of rat colonies and regular monitoring for this and for several viral agents is an essential precaution for all would-be "clean" rat breeding colonies.

b. **Bacterial Infections**: Severe clinical infections from bacteria are rarely a problem any more in rats. However, mild infections showing clinical signs occasionally occur and latent infections under stress may develop into clinical diseases.

Streptobacillus moniliformis may be present in the naso pharynx of apparently healthy rats and may infect bite wounds (usually to the hands) of persons working with them. The resultant Rat-Bite Fever is a severe systemic infection from blood-borne *S. moniliformis* (Anderson, Leary and Manning, 1983).

Corynebacterium kutscheri and *Streptococcus pneumoniae* are the causal organisms of pseudotuberculosis and pneumonococcal pneumonia respectively. Both are usually present only as latent infections but may flare up following stress or when complicated by other pathogens such as *M. pulmonis* in MRM.

Pseudomonas aeruginosa is ubiquitous in its distribution in conventional colonies but is not normally pathogenic. It is seemingly kept in balance in the gastrointestinal tract of rats by the other microflora normally present, and for this reason infections in barrier colonies may be particularly serious (Weisbroth, 1979).

Leptospera spp. that infect rats will induce only a transitory bacteremia at most. However, this organism settles in the kidney and is shed in the urine. Thirteen serotypes of leptospira have been identified in the rat and at least some of these may cause leptospirosis in man (Geller, 1979). This fact underscores the importance of washing one's hands after handling rats and not smoking or eating in the animal facility.

Salmonella spp. have not generally been considered a problem in rats in recent years and particularly not in barrier colonies. However, these organisms continue to be prevalent in wild rats and other rodents. Infections both in wild and domestic populations will most often be latent, with clinical signs becoming manifest under conditions of stress. The importance of surveillance is indicated by a recent report on a latent *S. enteritis* outbreak in a commercial barrier colony, which eventually led to the destruction of the whole colony of 35,000 animals (Steffen and Wagner, 1983).

c. **Viral Infections**: A majority of the viruses known to be naturally infectious for rats cause latent or "silent" infections. Their presence in overly healthy colonies can only be detected by serological monitoring (see discussion on latent virus infections in the chapter on Mice). Agents of the three virus families that are especially widespread among rat colonies will be briefly referred to:

   i. Parvoviruses—These DNA viruses are usually latent but may give rise to hepatic, vascular, and neurological lesions under conditions of immunosuppression (Jacoby, Bhatt and Jonas, 1979). Rat parvovirus has frequently been associated with tumours and recently a naturally-occurring rat parvoviral hemorrhagic syndrome has been reported (Coleman, Jacoby, Bhatt *et al.* 1983).

   ii. Coronaviruses—Two antigenically related RNA coated coronaviruses have been isolated from rats as the causal agents of distinct diseases, one of which, sialodacryoadenibs (SDA), is widespread and highly

contagious, although also both mild and transitory. Infection with SDA virus leads to an inflammation of the salivary and lacrimal glands. Photophobia, ocular lesions and bulging eyeballs with an overflow of porphyrin (red) tinged tears from the infected harderian glands occur, but usually subside after a week or two (Weisbroth and Peress, 1977). If the inflammatory reaction involves the salivary glands, it may lead to edema in the cervical region. A sub-clinical epizootic of SDA has been reported (Eisenbrandt, Hubbard and Schmidt, 1982). Rat coronavirus (RCV) infection is a related but distinct entity which is primarily pneumotropic, with little or no sialoadenitis being exhibited. RCV infections may prove lethal to neonatal rats but will almost be subclinical in animals over a week old (Jacoby, Bhatt and Jonas, 1979).

iii. Sendax virus (parainfluenza virus) causes a pneumonia in rats which is often associated with intercurrent infections with pneumonia virus of mice (PVM) and/or *Mycoplasma pulmonis* in MRM.

Spontaneous and experimental infections with Sendai virus alone have caused minimal clinical signs and are of low severity (Jacoby, Bhatt and Jonas, 1979; Castleman, 1983).

3. **Mycotic and Parasitic Diseases**

a. **Dermatomycosis**: Ringworm is seen less frequently in rats than in other rodents (mice, guinea pigs). The causal fungal species is probably always one or other form of the polymorphic *Tichphyron mentagrophytes*. The asymptomatic form of the disease is probably present more frequently than realized (this is particularly true of mouse colonies), and its presence may quite often pass unnoticed until a susceptible person contacts the infection. Treatment with griseofulvin in the feed or drinking water is sometimes successful but the recommended approach in most situations is to destroy the immediately affected group and to thoroughly disinfect everything with which they may have came in contact, before introducing new, clean animals (Weisbroth, 1979).

b. **Parasites**: Although the rat may harbour very many different ecto- and endoparasites, it is rare for any of these to pose a clinical problem in the properly run laboratory animal rat colony. In theory, parasites of any kind should be completely absent from barrier sustained colonies. In practice, however, occasionally one or other of several species of parasites may be introduced from conventional source animals or through contaminated feed and/or bedding; the more common of these are noted below:

i. *Syphacia* spp. are the commonly encountered pinworms of mice and rats. These small oxyurid nematodes are ubiquitous. Usually commensal inhabitants of the intestinal tract of clinically normal animals. *Syphacia* may be transmitted between different species of rodents, have a short, direct life cycle and if pinworm infestations are sufficiently massive they may jeopardize the validity of blood values and distort certain other data in critical behavioral and nutritional research (Kellogg Wagner, 1982). The presence of this parasite may be readily diagnosed by microscopic observation of their ova on a clear

cellophane tape anal impression. Once they become established, the control of oxyurid infestations is very difficult. Control measures should include sterilization of bedding, a rigorous campaign against outside rodents, use of filter caps and disinfection of equipment and all ducts (Harkness and Wagner, 1983).

ii. *Hymenolepis* spp. are dwarf tapeworms and are found in all rodents. The common species in rats are *H. diminuta* and *H. nana*, both of which are cestodes that may infect man and other primates. *H. nana* is the more serious zoonotic hazard as it is capable of a direct life cycle. These parasites are transmitted to and between rodents on contaminated bedding and by insects carrying eggs from one host to another (Harkness and Wagner, 1983; Hsu, 1979). *Cysticercus fasciolaris* is the larval stage of another adult tapeworm, *Taenia taeniaeformis*, that may also occasionally be encountered in laboratory rats, gaining entrance through bedding that has been contaminated by cat droppings (Harkness and Wagner, 1983; Hsu, 1979).

4. **Miscellaneous Health Problems**

a. **Neoplastic Disease**: Although spontaneous tumours develop in most strains of rats, particularly in animals of advanced age, the strain incidences are generally poorly documented. As is the case in mice, mammary tumours are the most commonly seen type, with some inbred strains having incidences approaching 50%. Neoplastic disease in rats has recently been thoroughly reviewed in terms of factors influencing tumourogenesis and the tumour type/incidence in various strains (Altman and Goodman, 1979).

b. **Alopecia**: The behavioral trait of "barbering" was described in the chapter on Mice. This dominant behavioral characteristic is also occasionally encountered in group housed rats and should be differentiated from alopecia due to other, usually more serious, causes (Bresnahan, Kitchell and Wildman, 1983).

c. **Allergy**: A survey on problems in personnel resulting from association with laboratory rats in the USA and Canada revealed that 23 of 42 responding institutions had personnel who had encountered various allergic reactions to rats. Most sensitive individuals had a personal or family history of allergy (Geller, 1979). In employment situations involving sensitization to laboratory animal danders, the most frequent allergen source appears to be the rat (Lutsky and Neumann, 1975).

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