Notes for breeders of common laboratory animals / edited by George Porter and W. Lane-Petter.

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NOTES FOR BREEDERS OF COMMON LABORATORY ANIMALS

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Edited by

GEORGE PORTER and W. LANE-PETTER

M.R.C. Laboratory Animals Centre
Carshalton, Surrey, England

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Foreword

The contributors to this book include graduate scientists, technicians and commercial breeders. They have one thing in common, an intimate practical knowledge of breeding and keeping the more frequently used laboratory animals, and they have treated their subject in a strictly practical way.

It was in 1950 that the Laboratory Animals Centre (or Bureau as it was then called) introduced throughout Great Britain an accreditation scheme for breeders of guinea-pigs, mice and rabbits, the three species whose supply to laboratories was largely met from commercial sources rather than from laboratory breeding. This scheme had a twofold object; to help laboratories to pick out the primary breeders who were able to observe high standards of hygiene and management in order to produce good animals, and to find out whether such breeders were able to improve their standards even further.

The first object was quickly attained, and over the years most laboratories have found it wiser to buy their animals from accredited breeders rather than from non-accredited sources. The second object entailed an educational programme in order to put a little science into traditional practice, and an important element in this programme was the production of a magazine, called the *Laboratory Animals Bulletin*, written in simple language by people who had the necessary knowledge. The first *Bulletin* appeared in September 1950 and the fifteenth and last in January 1956. Between them they covered the whole field, and so the series was discontinued.

The *Bulletin* had a warmer reception among breeders than the compilers had ever dared to hope. Moreover, it found its way into the laboratories and, when publication ceased, the demand nevertheless continued. In 1957 a consolidated edition, in mimeograph, was produced, containing the best of the articles published in the fifteen separate numbers, and this has had to be replicated on two subsequent occasions. Yet it is still requested, and so it was decided to publish it as a printed volume.

The present book is the result. All the articles have been carefully selected and brought up to date. New ones have been added to enhance the general interest. Breeders should find here much that will help them to meet the high standards that the laboratories demand of them.

Users of animals may also come to see the difficulties of the breeders. Perhaps one of the best features of the Laboratory Animal Centre's accreditation scheme is that it has helped breeders and users to get to know one another, and to learn about each other's problems. Some of the results of this acquaintance are contained in the papers that follow.

CARSHALTON, 1962

G. PORTER

W. LANE-PETTER

I

Guinea-Pigs

1. NOTES FOR THE GUIDANCE OF BREEDERS OF GUINEA-PIGS FOR LABORATORY USE

A. J. H. Tomlinson

It must be emphasized that guinea-pigs will breed and thrive under a wide variety of conditions. It is not good policy to lay down exact conditions under which animals must be kept, since these will vary; much can be learnt by observation and improvements suggested by comparison with other breeders. Certain points, however, must be attended to with meticulous care, otherwise failure is almost certain.

(A) Housing

The house in which the animals are kept must be free from infestation by rats and mice. Most guinea-pig breeders, for obvious reasons, take steps to keep rats out, but mice are a commoner pest and less easy to eradicate. The danger from mice is the possibility that they may be infected with *Salmonella* or *Pasteurella* organisms (germs) and carry this infection to the guinea-pigs. In both cases the infecting organism is present in the droppings so that any contamination by mice in the house or in the food store is a potential danger. It is of no value to catch the mice in the house, they must be prevented from entering. The actual method used depends on the house in question. A wooden house should be raised on bricks, all possible holes blocked up, or if this is not possible, fine wire gauze nailed over them. In stone or brick buildings it is necessary to prevent the entry of mice into the space between the walls and to prevent their exit from here into the animal house. Doors and windows should be made to fit closely. For mice already in the buildings trapping is to be preferred. Poisoning with chemical poisons may be used with care, but on no account should the "virus" poisons be used, since these often consist of preparations of *Salmonella* organsims.

The house, of course, must be dry and sufficiently well ventilated to carry off the moisture exhaled by the animals. During the winter, attempts to conserve heat by closing the ventilators will lead to condensation of this moisture, making the building permanently damp and

often damaging its fabric. Adult guinea-pigs will survive the winter in unheated houses, but breeding under these conditions, particularly in some areas, is not certain and the chance of rearing the young remote. Laboratories require a constant supply of animals during the winter and often the demand decreases in the summer owing to holidays. Successful management demands that breeding should continue throughout the winter at the maximum rate and, therefore, some form of artificial heating has to be supplied. The aim must be to maintain a steady temperature of 55-60°F with adequate ventilation: the actual method is immaterial. For the larger house, circulating hot water (as in a greenhouse) may be used; for the smaller house, electric heaters may be satisfactory. The latter, while the most convenient, is probably the most expensive to run and the tendency is always to use too little.

(B) Management

The larger the groups in which guinea-pigs are kept, the more difficult it becomes to control infection. Ideally, therefore, three to six sows should run with one boar and larger groups should be avoided. The same principle applies to the running on of young stock recently weaned. If kept in small groups, all do equally well, but if large numbers are put together, the weaker animals will not grow, probably because they are unable to compete for food. If large numbers of young animals are run together, an infection can spread among them with great speed.

In some establishments the sows are allowed to have their litters in the pen with the boar and the other sows of the group. This method has both advantages and disadvantages. The sow is mated as soon as she has produced her litter so that another pregnancy is started without delay, but this only happens in about two-thirds of the animals. The disadvantages of this method are that losses of newly born young are higher than they should be owing to the attention of the boar to their mother, the eldest litter in a pen will often strip the milk from all the sows, leaving little for the other litters, and it is very difficult to keep a check on the performance of the individual animals.

In other establishments the sows are transferred to separate small cages when pregnancy is well advanced. The advantages of this method are that the young get the whole-time attention of the mother and all the milk that she provides. It is easy to keep a check on the performances of the mother and to provide additional food where necessary. The disadvantages are that the average yearly number of litters is

reduced from five with the first method to about three and a half with this method, the space required for a given number of sows is increased by about 70% and the labour involved in looking after the animals is increased. Some breeders find, however, that the additional number of animals reared per litter makes this the method of choice.

Other breeders try to combine these two methods by removing the boar when the sows are obviously in pig. If care is taken to avoid more than one litter in a cage at one time, it is possible to combine the higher productivity of the second method with the economy in space given by the first method.

The female guinea-pig should not be mated before she is 4 months old. The first litter produced is usually rather smaller in number, and the number born dead is higher than in subsequent litters. The next five or six litters are the largest, and thereafter the numbers decline, but the actual decline differs in different animals. It is important, therefore, to watch the performances carefully and to cull unproductive animals from the herd. Although the young born in small litters grow fastest, it is a mistake to use these animals for breeding since the characteristic of small litters is thereby perpetuated.

Any female that does not rear to weaning at least ten young per annum should be discarded. This means that in polygamous herds (where the boar runs with the sows all the time) at least two and a quarter young must be reared, on the average, per litter, and when animals are separated or the boar is removed for littering at least three per litter. These figures can easily be attained by well-fed healthy stock.

It cannot be too strongly emphasized that the guinea-pig herd must be kept as a self-contained unit and no new stock introduced from outside. Breeding within the herd should not lead to any deterioration in the stamina or fertility of the stock. If it is desired to introduce a different colour or other characteristic into the herd, then animals from an outside source will have to be introduced, but it is important that before this step is taken the opinion be obtained of some competent authority, who will be able to advise on a safe source from which to obtain these animals. It is safer to obtain males since these are not so likely as females to break down if chronically infected, and they should be kept in quarantine for some weeks before being admitted to the herd. When they have mated some sows, it might be safest to dispose of them. On no account should adults be introduced into the herd merely to increase the numbers of breeding animals. If an increase in numbers is desired, young stock must be kept from the produce of the herd. This

means that it takes at least six months before the output of the herd can be increased, but the danger of the introduction of infection is avoided.

(C) Diet

Guinea-pigs can be reared on a number of different diets. A mash of bran and oats or bran and sugar-beet pulp may be given, or alternatively a more balanced pellet diet may be used. The latter is more economical, as less of the food is wasted; and since a pellet diet may be fed from a hopper, labour may also be saved. Too much food should not be given at one time since this leads to spoilage and waste, but sufficient food must be provided.

In addition to mash or cereal, hay and green food must be given. Hay is of importance in the provision of bulk in the diet; healthy animals will clean up completely all the hay they are given provided it is of good quality. Green food, particularly the leaves and stems of brassicas (cabbage, cauliflower and kale), is essential as a source of vitamins and of other substances essential for healthy animals.

During the winter many breeders feed root vegetables instead of green stuff. Some animals, particularly non-breeding adults and young stock, will survive on this diet; some sows will rear a litter on this diet, but if the herd receives root vegetables instead of green food, losses of adults and young are higher, partly from dietary deficiency and partly from infection, and the condition of the whole herd deteriorates. It is essential for the successful management of the herd to keep the animals in first-class breeding condition throughout the year and to provide green stuff in the winter, either by purchase or by horticulture.

Water must be offered in some form. Provided green food and wet mash are given, it is probably unnecessary to give additional water. Some breeders find that animals given water grow faster and rear more of their litters than animals without it. This difference is more marked in warm houses, and it is probable that well-managed herds in heated houses, breeding throughout the winter when green stuff is scarce, will derive benefit. If a pellet or dry diet is fed, water is essential.

(D) Disease

In a herd of healthy well-fed animals, deaths except by accident are exceptional and should be less than 1% of the adult stock per annum. Although up to 8% of the young born in first litters may be

born dead, the proportion in later litters is lower. Deaths of healthy guinea-pigs more than 24 hours old are exceptional except in litters of five or more, when lack of food may be a contributory cause. Abortion is often a sign of infected stock.

Most of the deaths in guinea-pig herds are due to infections, and these can be divided into two groups. The first group consists of respiratory infections that are relatively unimportant, since they do not spread to any extent in healthy well-fed herds. Included in this group are infections caused by *Pneumococcus*, *Streptococcus* and *Haemophilus bronchisepticus*. These infections are primarily of the lung, they occur most often in the winter months and are encouraged by overcrowding, cold and a deficient diet. The remedies consist of ensuring adequate space, an adequate diet and adequate warmth.

The serious infections are those caused by organisms of the *Pasteurella* and *Salmonella* groups. When one of these is introduced into a group of healthy animals there is a widespread epidemic with a high death-rate, and up to 70% of the animals in the herd may die of an acute infection. Some of those that survive will develop a more chronic type of infection and may appear to have recovered completely. A sudden change of diet or of temperature, an inoculation, a railway journey or the strain of littering may upset the balance between the infecting organism and the animal and lead to the death of the animal. These infections enter the body through the mouth and the organisms are present in the droppings, particularly if the latter are liquid. Chronically infected animals may not be infective while they appear in good health, but they excrete the infecting organisms in large numbers in the few hours before they die and thus infect other animals. Young animals born into an infected herd will become infected during their early weeks; some will die of the acute infection, others will survive in the chronically infected condition and others again will show no signs of infection at any stage. Animals that appear to be perfectly healthy may be infected and may be able to pass the infection on to others.

In addition to causing the death of young and adult animals, *Salmonella* infections may lead to abortion; adults, once infected, tend to be barren or to produce dead litters. The diagnosis can only be made in the laboratory after death. No treatment is of any avail and there is no test of value in determining which animals are healthy and which are chronically infected.

It follows, therefore, that prevention is the only useful method of dealing with these infections. *Salmonella* and *Pasteurella* are naturally

present in rats and mice and these animals must be kept out of the guinea-pig house. In addition, *Salmonella* will infect man and all species of domestic animals, including hens and rabbits, and it is necessary to keep the guinea-pigs carefully segregated from these animals. The most potent source of infection is other guinea-pigs introduced into the herd, and this course must be undertaken only after the most careful thought and tests to ensure the healthiness of the introduced stock. A sure way of picking up infection is to buy animals from several different sources.

It should be realized that the exhibition of animals at shows is a possible source of infection, and the careful breeder, if he must show his animals, will never return them to his breeding herd, but keep them in a separate house away from the others, or get rid of them altogether.

As a further precaution it is desirable to keep the guinea-pig herd in a number of separate self-contained units. Should an infection start in one of these units it may be possible to localize the outbreak and the breeder will have healthy stock with which to build up again.

The management of a herd in which these infections have been established is a difficult and unrewarding task. Assuming that healthy stock can be obtained, that the buildings are suitable, and that there is a reasonable chance of keeping the animals free from infection, it is best to kill off all animals, burn the cages if made of wood, scrub, scrape and disinfect the house and start again from a small number of healthy animals. If this is not possible, attempts must be made to keep the infection under control by adequate diet, avoidance of overcrowding, splitting the herd into smaller units and rigorous culling of animals that do not appear to be in the best of health. It is unlikely that these measures will eradicate the infection, but they may keep it under control.

(E) Conclusion

There is no doubt that guinea-pigs from small breeders can be of very high quality from the point of view of laboratory work, but it is essential that they be healthy and well fed. Infected, ill-fed stock is unlikely to find a ready sale in the future. A serious attempt must be made to produce animals throughout the winter since this is the time when most laboratories are busiest. The demand in July and August is always low. Regular orders can only be given to those who produce animals regularly, and those breeders who only produce animals in the summer may expect to find difficulty in disposing of their stock.

It is to the advantage of both parties that the stock should be healthy and adequately fed. It costs the breeder less to produce healthy stock, the same amount of labour is required to look after healthy as after unhealthy stock, but it is much more satisfsctory to keep healthy stock, and the financial return on it is greater.

The laboratory using the guinea-pigs will be prepared to examine animals dying naturally and to advise on the management of the herd and the methods to be undertaken to deal with established infection. If the laboratory using the guinea-pigs is too far away, these duties may be delegated to some more conveniently situated laboratory. It is desirable that the closest collaboration be maintained between the laboratory and the breeder, since considerable mutual benefit can accrue to both parties. Where possible, the breeder should endeavour to discover what is likely to be the demand for animals in the future so that stocks can be built up to meet an expected increase in demand, or sows rested when the demand is falling off.

2. THE PRODUCTIVITY OF ACCREDITED HERDS OF GUINEA-PIGS

A. J. H. Tomlinson

The present high price of feeding-stuffs and the consequent high price of guinea-pigs are of concern both to the laboratories using animals and to the breeders producing them. The only practicable solution of the difficulty at the present time is to assist guinea-pig breeders to produce as many animals from their herds as is compatible with the maintenance of quality. It is possible to obtain from publications and other sources many different opinions about the best methods of breeding and raising guinea-pigs, but few of these statements are based on more than the authors' impressions and actual figures are seldom given in support of them; in addition, many of these methods are designed to produce animals for exhibition and are therefore quite different from the methods needed to breed guinea-pigs for laboratory use.

It seemed that useful results might accrue if information was obtained from a number of breeders about their methods of management and feeding, and these compared with the productivity of their herds. Productivity was defined as the number of young, reared to weaning, per adult sow per annum. Accredited breeders were selected, since their

herds were believed to be free from serious infection and they were most likely to have records from which the productivity could be determined. A letter and a questionnaire were sent to a number of accredited breeders and the following results are based on their replies. I am very grateful to all those who have been able to supply information, since without their co-operation no results could have been obtained.

(A) Results

Figures for productivity were obtained from ten different breeders; these were based on periods of between 6 and 12 months and in each case were worked out to give the productivity per sow per annum. The productivity varied between 4·2 and 12·1 per sow per annum; three breeders had less than 6, three between 6 and 10 and four over 10 per sow per annum.

Many breeders were increasing the size of their herds during the period under review; the results were not obtained from stable herds and must include a high proportion of first litters. There is also the possibility that some inaccuracy in the records may have made the productivity appear slightly too low, nevertheless it is clear that a number of herds have less than half the productivity of the most successful breeders. It is also clear that a productivity of 10 is within the reach of all breeders and that this figure can be taken by breeders as a convenient standard by which to judge their own performance.

An attempt has been made to correlate the diet and management of the different herds with their performance and to determine which factors are of the greatest importance. These results must necessarily be given in general terms, since many factors are involved. The composition of the diet seemed to have little effect on productivity, good and bad results being obtained on the same diets. All breeders fed hay daily and all fed green-stuff over as long a period as possible; the best results were obtained by feeding some green-stuff daily throughout the winter. Those breeders who had to fall back on root vegetables as the only fresh food did not do so well.

Only one breeder ran the boar with the sows all the time; he had the highest productivity of 12·1 per sow per annum. Five of the others removed the sows to separate pens before they littered, and four allowed two or more sows to litter together, the boar having been removed. There were considerable differences between individual herds in each of these two groups, but in general better results were obtained by those whose sows littered alone—presumably because

their losses after littering were smaller. One breeder had better results with two sows littering in one pen than when the sows littered alone, but this may be due to other factors.

Productivity was affected to a considerable extent by the temperature at which the herd was maintained during the cold weather. It is impossible to assess accurately from a questionnaire the temperature conditions prevailing in different breeders' houses since these must depend on situation, type of building and many other variables, but it was clear from the answers that those breeders who maintained, as far as possible, a steady temperature throughout the winter obtained the best results. Those who gave no artificial heat and those who only gave some heat during the very coldest weather did not do so well.

The young were weaned in all cases between 2 and 4 weeks after birth; the most successful breeders did not find it necessary to rest their sows after the young were weaned, but others rested their sows from 2-3 weeks before they were returned to the boar. (This may be an indication of a diet insufficient in quantity during pregnancy and lactation.) It follows that any undue delay in returning the sow to the boar after littering will lead to a fall in productivity. To maintain productivity the young should be weaned as soon as possible and every effort made to keep the sows in good condition.

Most of the stocks of guinea-pigs outside institutions are derived ultimately from exhibition stock. For exhibition purposes small litters are desired and in consequence these stocks are not very productive when bred for laboratory use. It is of interest that the most successful results have been obtained by breeders using stocks selected for high productivity, either by themselves or others, over a period of years. That this does not involve any loss of quality is shown by the size and rate of growth of such stocks.

Intensive production of any livestock is difficult and requires more care and a better diet than less intensive methods if it is not to lead to increased susceptibility to infection and to loss of quality, and this is true of guinea-pigs as of other animals. If the boar and sows are run together all the time, a high productivity is attained. The effective reproductive life of the sow is short, probably not more than 2 years from the time of first mating, and it is widely believed that this method leads to a loss of quality. When the sows are removed from the boar before littering, the effective reproductive life of the sow is greatly increased and if the sows litter singly the losses after birth are very low. Fewer litters are produced in a year by this method and unless the

average litter is large, the sows maintained in good condition and mated soon after the young are weaned, the productivity will fall. Guinea-pig stocks selected for large litters will average at least three young reared per litter (including all dead litters), half the litters will be of four or more and one litter in five will be of five or more young. Unless the stock can come up to this standard, productivity will fall below an economic level if the sows litter away from the boar. Nevertheless, unless the productivity of the less successful breeders can be increased in other ways a change to a more intensive system may be the only way of making the herd successful economically.

In such an intensive system it is best to breed a few selected sows less intensively to obtain replacements for the breeding stock.

It is possible by selective breeding to perpetuate in livestock almost any good or bad characteristics. In guinea-pigs for laboratory use one requires sows that thrive easily, produce large litters regularly without difficulty or still-births, and whose young grow rapidly. In the selection of breeding stock these are the points to watch. Although the boar does not influence the size of the litter he gets, he has a profound influence on the productivity of his daughters. Unless the greatest care is exercised in the selection of stock to be retained for breeding, the productivity of the herd will fall; but successful selection over a period will lead to better results.

Breeders should keep for their own information a certain minimum of records: such records will add greatly to the interest of keeping guinea-pigs and will show the breeder, more certainly than any visiting expert, when and where conditions are not optimal. Productivity should be constant throughout the year: if it is found that productivity falls in the winter and spring, then some improvement in the conditions prevailing during the winter is required. Certain sows may have small litters or some of their young may fail to grow as fast as others: such sows should be removed from the herd. Other sows may have unusually good records and it is from these that breeding stock should be selected. Another record that is very valuable is the date on which a particular sow was returned to the boar: if that sow does not produce a litter until 15 or 20 weeks later, something is wrong. This is particularly likely to happen in the winter and early spring unless adequate warmth is provided. Another reason for keeping records is that there are many points in the breeding of guinea-pigs about which too little is known and the answers will only come from a careful study of the records of individual breeders.

It is clear that many breeders are not getting the best results from their herds. Everything possible must be done to improve this situation, since the financial disadvantage imposed on a breeder by low productivity may be too great to allow him to continue in business. The results given here may be of some assistance in showing breeders which factors are of the greatest importance.

3. POST-PARTUM BREEDING IN THE GUINEA-PIG

I. W. Rowlands

When the guinea-pig is bred by the ordinary method (separation of the sexes before parturition), an interval of about 15 weeks elapses between the birth of succeeding litters. In other words it takes a sow 2 years to produce seven litters. It is not very widely realized that the guinea-pig, in common with several other laboratory rodents, has a period of "heat" and will mate within a very few hours after farrowing. This period is known as the post-partum "heat". Thus, by the usual method of breeding, the chances of mating occurring at this "heat" are lost. If a sow farrows in the presence of a boar, there is a good chance that another pregnancy will be well advanced by the time she has reared her previous litter and another litter will be born 63-70 days later. This method of breeding reduces the interval between the births of two succeeding litters from 15 to about 10 weeks. A healthy sow should be capable of producing five litters in a year.

Bruce and Parkes, using guinea-pigs of the M.R.C. strain kept continuously together in pairs, found that 74% mated at the post-partum "heat". The young were healthy and strong and the breeding sows took the strain of this intensive breeding without any ill-effects. Pair-breeding cannot be applied economically on a very large scale because of the large numbers of males that would have to be maintained, and to overcome this disadvantage I tested the possibility of post-partum breeding in small colonies of animals in floor pens in a heated room, and the results obtained are described below.

(A) Design of the Test

A number of small floor pens 7 ft by 3 ft 6 in. were built with asbestos sheeting, the walls being 18 in. high. Variable numbers of animals, as shown in Table I, were placed in these pens, in some of which post-partum mating was allowed by keeping the males continuously with the females (Method 1). Other pens were fitted with a

partition to separate the pregnant sows from the boars. In these pens, post-partum mating did not occur; the sows were returned to the boar for re-mating after all the previous litters had been weaned and removed from the pen. This method (Method 2) is comparable to the usual method as carried out in hutches or cages and referred to as Method 3 in Tables II and III.

All the females in a pen were given an identification mark (stain) and the young at birth were given the same mark as the dam. The dates on which litters were born were recorded; litter-size, and the numbers of still-born and viable young were noted. The latter were weaned when 4 weeks old. All deaths up to the time of weaning were accurately recorded.

The guinea-pigs used in this breeding experiment were reared from stock originating from the M.R.C. strain. They were fed daily with 1 oz of Diet 18 (M.R.C.), 3 oz of green-stuff, and a plentiful supply of hay and water. The test was carried out for a period of about 17 months so that seasonal changes in breeding performances could be ascertained and the effects of a limited supply of green-stuff in the winter and spring months could be investigated.

(B) The Time of Occurrence and Incidence of Post-Partum "Heat"

It is difficult to obtain direct information on the time at which mating occurs following parturition because the majority of litters are born during the night and mating will have occurred by the time the sows are examined the following morning. This is confirmed by the finding of a vaginal plug. However, in three cases in which parturition and mating were observed the interval between these two events was $1\frac{3}{4}$, $2\frac{1}{4}$ and 3 hours respectively. These three sows gave birth to litters 68 days later. Further information on the time and incidence of post-partum mating and the gestation period that followed was obtained by separating sixty sows from the boars on the morning that litters were first noticed in the pen. These sows were not replaced with the males until one week later. Forty-five (75%) of these sows farrowed within a period of 70 days, the average period being 68 days. None of the remainder littered before the 88th day, which indicated that mating had not occurred until after the sows had been put back with the boars. This experiment gives some indication of the proportion of sows that mate with fertile results at the post-partum "heat". A litter born within 70 days of the birth of the previous litter is considered to have been conceived at the post-partum "heat".

A study of the records of individual sows breeding throughout the 17 months of this test has shown that only one sow failed to conceive once at the post-partum "heat" during the entire length of her breeding life. On the other hand, every litter of fifteen sows was conceived at a post-partum mating, and of these sows, eight conceived at seven consecutive post-partum "heat" periods and two at eight consecutive post-partum "heat" periods.

(C) Post-Partum Mating and Size of Colony

Table I gives the proportion of fertile post-partum matings in colonies consisting of variable numbers of sows and boars. In most of the colonies all the matings were effected by one boar, but in two pens a second male was introduced.

Table I. Fertile Post-Partum Matings in Relation to Colony Size

Size of colony			*No. of litters born*	*No. of fertile post-partum matings* (%)	*Mean interval between birth of succeeding litters* (*days*)
No. of females		*No. of males*			
5	:	1	31	87	68·6
10	:	1	58	83	73·6
12	:	1	12	83	69·0
15	:	1	94	73	74·8
20	:	1	39	52	76·0
10	:	2	53	83	71·6
15	:	2	78	73	76·6

It will be noticed that the number of post-partum matings decreases in proportion to the number of females in the colonies having only one male. One male is capable of mating only about one-half of the females in a colony of twenty. The addition of a second male to a colony of ten or fifteen sows does not raise the proportion of fertile post-partum matings. This confirms observations made on the behaviour of the two males to each other. One male becomes dominant and prevents any attempts by the other to mate a sow. It is likely that the dominant male is responsible for all the matings in the pen and this behaviour, therefore, sets a limit to the size of the colony that can be used. The optimal size of colony is considered to be twelve sows and one boar.

The figures given in the right-hand column of Table I are the mean intervals between all litters born irrespective of the occurrence of post-partum mating. The average interval between the births of succeeding litters of all the sows is about 74 days. In three pens in which sows were separated from the males to prevent post-partum

mating (Method 2), the average interval between births of succeeding litters was 118 days. Thus a saving of 44 days is effected between the births of litters by allowing post-partum mating to occur.

(D) Litter Size and the Survival of Young Guinea-Pigs

The records made on litter-size and the survival rate of the young are summarized in Table II. They include data taken from breeding by another method (Method 3) which for all practical purposes is similar to the usual method of cage breeding in which the pregnant sows are removed to separate cages for farrowing and rearing their litters. The difference between Methods 2 and 3 is that whereas in the latter the sow rears her own litter in isolation, in the former the young are born on the floor in the same pen as others and they are suckled by any lactating sow.

Table II. Litter Size According to Breeding Method.

Breeding method	*No. of litters born*	*Average litter size*	*Live births* (%)	*No. of young weaned* (%)
1	406	3·98	95·6	84·7
2	139	3·82	94·7	86·0
3	234	3·69	95·8	93·9

Method 1 = post-partum. Method 2 = post-lactational breeding in colonies.
Method 3 = post-lactational breeding and rearing in isolation

Of the 406 litters recorded for Method 1, 80% were conceived at post-partum mating and contained an average of 4·15 young, the remainder contained 3·93 young apiece. The latter figure is comparable with that given for Method 2 (3·82). It seems, therefore, that slightly larger litters are conceived at the post-partum "heat", but from the practical point of view the difference is so small as to be of negligible value.

Live births amount in all three methods to 95%. This implies that 5% of all young are still-born. The main cause of death at birth is suffocation due to the sow failing to release the young from the foetal membrane. These deaths occur more frequently in large litters.

The last column of Table II indicates the proportion of young that are successfully reared. The difference between these figures and 100% represent the death-rate sustained, including the still-birth rate. As the still-birth rate is about 5% by all three methods of breeding, it can be shown that the death rate amongst young guinea-pigs between birth

and weaning is about 10% for Methods 1 and 2 and is negligible for Method 3. It is clear, therefore, that this high death rate in the former methods must be attributed to the colony system and not to post-partum breeding. The majority of deaths result from trauma caused as the result of the unavoidable stampeding which occurs when the animals are frightened by noise within the colony.

The death rate among young tends to be higher in the winter and spring months when fresh green vegetables are scarce.

(E) Duration of Post-Partum Breeding

It was intended originally to allow the sows in this experiment to breed until sexual activity came to a stop in order to investigate the overall reproductive capacity of the guinea-pig. However, after 17 months of breeding the experiment was brought to a sudden standstill by an outbreak of infectious disease (*Salmonella typhimurium*—rat typhoid) which necessitated the destruction of all the animals. Until this outbreak arose the animals remained healthy and showed no obvious strain from this intensive breeding method. The outbreak could not be attributed in any certain degree to the lowering of the animals' resistance to disease, but as a precautionary measure it is recommended that every sow should be removed from breeding after the weaning of her fifth litter. This implies that a sow is breeding continuously for about one year before being discarded.

(F) Annual Production of Young by Different Breeding Methods

An attempt is made in Table III to assess the average production rate of a sow during one year of breeding by the three methods.

Table III. Annual Production Rate of Guinea-Pigs

	Breeding method		
	Method 1	*Method* 2	*Method* 3
Interval between births of succeeding litters (days)	74	118	105
Calculated number of litters/annum	4·9	3·1	3·5
Average litter size	3·98	3·82	3·69
Calculated number of young born/annum	19·5	11·8	12·9
Weaning rate (%)	84·7	86·0	93·9
Number of young reared/sow/annum	16·5	10·1	12·1

The table shows that the production rate is greatest by Method 1 (post-partum mating) and is largely due to the much more rapid rate of littering. The body weights of the young animals conceived at

post-partum mating are similar to those of others bred at the slower rate (Methods 2 and 3). The post-partum method has now been used for some years in the large colonies on this Field Station and there is no indication of any deterioration in the quality of the young which are produced.

(G) Conclusions

The main points that have emerged from these observations on post-partum breeding in the guinea-pig are as follows.

1. The average gestation period is 68 days.
2. About 80% of all matings were effected at the post-partum "heat".
3. The number of fertile post-partum matings decreases appreciably when the proportion of females to males in the colony is greater than 15:1. The optimum proportion is considered to be 12 females and 1 male.
4. The interval elapsing between the birth of succeeding litters in a pen containing the optimum proportion of the sexes is about 70 days.
5. When post-partum mating is not permitted this interval is 118 days.
6. A still-birth rate of 5% is recorded, the deaths being caused by suffocation of the young within the membranes. This death rate is not attributed directly to the method of breeding.
7. The death rate among young animals reared communally in colonies on the floor, is greater than that occurring amongst others reared in cages. The higher death rate in the former is not attributed to post-partum pregnancies.
8. The annual production rate is greater when animals are bred at the post-partum "heat".
9. The body weights of the young animals bred by the post-partum method are similar to those of others bred by the more usual methods.

4. COMMERCIAL GUINEA-PIG BREEDING

R. Blyth

There are probably as many different methods of breeding guinea-pigs as there are breeders of guinea-pigs, and almost as many different ways of housing them. Before going on to describe one particular system it would be, perhaps, of some interest to examine those factors which influenced the writer in his choice.

Apart from those who keep guinea-pigs merely as pets, the breeder usually has one of two objects in view; either he is a fancier breeding for show or he is a commercial breeder producing animals for the laboratory markets. These words are not addressed to the former, nor to the optimist who stands precariously with a foot in each camp, but

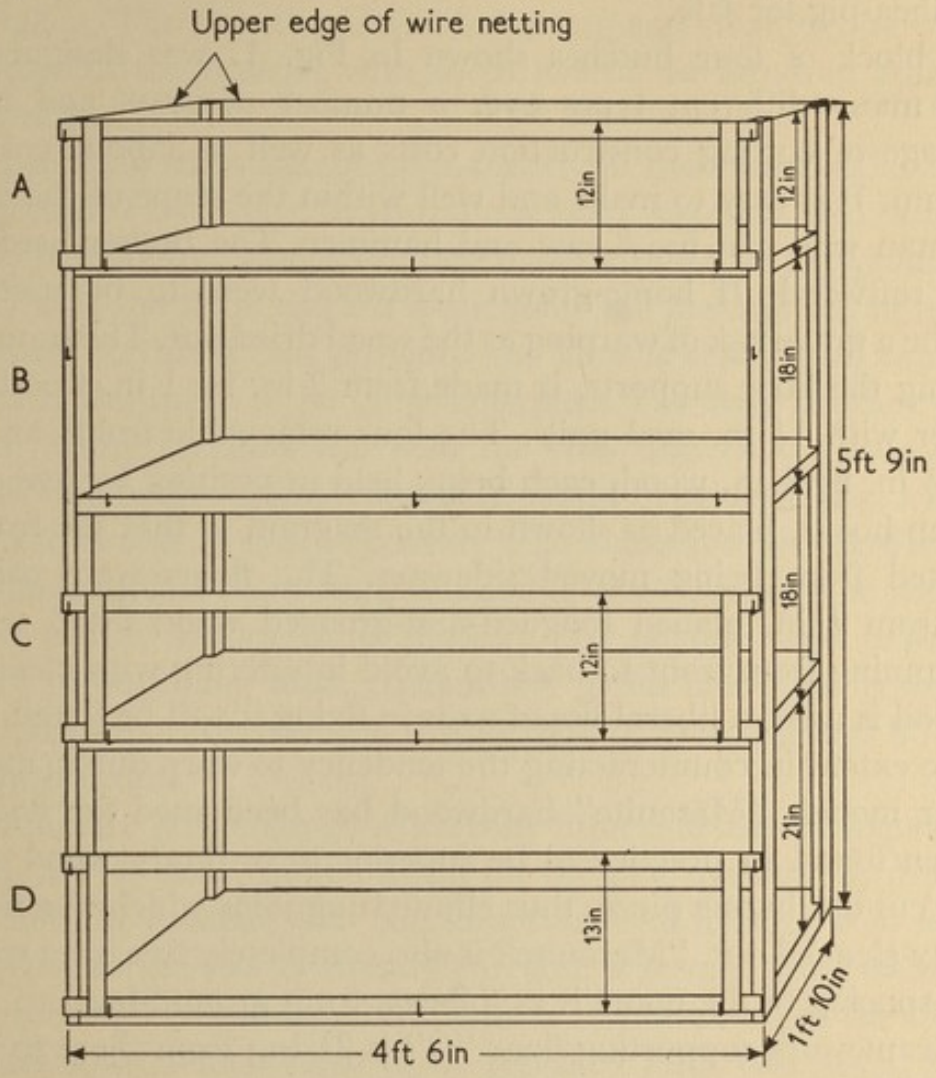


Fig. 1.

to him whose sole object is to supply the laboratories with their requirements. The fancier who does read on will no doubt be horrified at what is to follow, but as the end in view differs from his, then the means to that end must also be different. The commercial breeder's concern is to produce healthy animals at an economic price—economic both from his own standpoint and from that of the purchaser. Obviously it would be useless to produce healthy specimens at a price which put them beyond the reach of his prospective customer, and altogether futile to produce unhealthy animals. Whatever system of breeding is adopted, the stock must be well housed, well fed and clean at all

times. Keeping this in mind, production costs must be kept to a minimum without sacrificing quality. By reducing housing and labour costs whilst at the same time increasing the number of young reared per sow per annum, the breeder can most effectively influence that figure which is the measure of his success or failure, i.e. the cost of producing one guinea-pig for sale.

The block of four hutches shown in Fig. 1. was designed after testing many different types over a number of years and has the advantage of cutting construction costs as well as labour costs to a minimum. It is easy to make and well within the scope of the average handyman who can use a saw and hammer. The timber used is imported softwood. If home-grown hardwood were to be used there would be a grave risk of warping as the wood dried out. The framework, including the floor supports, is made from 2 in. by 1 in. wood nailed together with $1\frac{1}{2}$ in. oval nails. The four removable fronts are made from $1\frac{1}{2}$ in. by $\frac{1}{2}$ in. wood, each being held in position by five square-type cup hooks, placed as shown in the diagram so that the fronts are prevented from being moved sideways. The floors were originally made from $\frac{1}{2}$ in. planed tongued-and-grooved wood fitted with the joins running from front to back to avoid interfering with cleaning. If this wood is used, a liberal use of nails in fixing it will be found to help to some extent in counteracting the tendency to warp due to moisture. In later models "Masonite" hardwood has been used for floors and has been found in practice to be superior to ordinary wood as each floor is cut out in one piece, thus eliminating joins which can never be properly cleaned out. "Masonite" is also completely free from warping.

The spacing of the floors is as follows: from ground-level to the top of the framework supporting floor "C" is 21 in.; from there to the top of floor "B" 's supports is 18 in.; from there to the top of floor "A" 's supports is also 18 in., leaving 12 in. to the top of the block. The hutch fronts are 12 in. deep with the exception of front "D", which is 13 in. The reason for this is that the animals on the bottom floor are more likely to attempt to get out than are those on the upper floors. Floor "B" is shown with the front removed. The whole of the two sides and rear of the block, together with the four fronts, are covered with $\frac{1}{2}$ in. mesh wire netting. This has been omitted from the diagram for the sake of clarity. The netting is fixed to the outside of the fronts in order to prevent it interfering with their fitting. The single line round the two sides and back of the block at the extreme top represents the upper edge of the wire netting. It will be seen that when the block is assembled

the top of hutch "A" is completely open while there is a 5 in. feeding space above the fronts of each of the other three hutches. In addition to feeding, these gaps are also used for the removal of pigs. The only occasion on which the fronts are removed is when the floors are cleaned. This can be done without removing the animals, provided reasonable care is taken. The time thus saved in feeding and cleaning, particularly in the case of the breeder who keeps five or six hundred animals, is considerable.

The breeding stock is kept permanently in these hutches, each floor being occupied by twelve sows and one boar, together with a number of unweaned young. Two objections may be raised at this point. Some may say that the floor space is inadequate for the number of animals. The answer to this is that provided the floor is kept clean, experience has proved the space to be quite adequate. The only bedding used is sawdust. Many breeders will hold the view that twelve sows are too much for one boar. Again, it has been found in practice that a healthy young boar is able to deal effectively with a dozen sows. Here it should be emphasized that plenty of good food, given frequently, is absolutely essential if this system is to succeed. To anyone who experiences difficulty in obtaining food, particularly green food, and is forced to use a high proportion of roots, this method of breeding and housing is *not* recommended.

The sows are not removed when they litter and are consequently, in the majority of cases, mated again on the day the litter is produced. It will now be evident why the importance of feeding is so strongly emphasized as the sow is already carrying a second litter while she is feeding the first. Naturally, this intensive system shortens the breeding life of the sows, which have to be replaced after two years, but the increase in production makes this well worth while. Some breeders are of the opinion that young animals born under these conditions sometimes suffer damage as a result of their elders trampling on them. This drawback has not been experienced; on the contrary, during a birth the animals usually assist each other to clean up the young. Even on those frequent occasions when a birth takes place during feeding time, with the inmates of the hutch in a state of excitement, no harm is done to the new arrivals. The boar never interferes with them at any time.

The writer does not claim that this system of breeding is perfect, but only that it is far better than any of the previous methods he has tried. The above description may be of interest to other breeders, and if perchance it evokes some constructive criticism, then perhaps another

step forward may be taken towards the goal of evolving an ideal system of commercial guinea-pig breeding.

5. BREEDING TO A PLAN

S. G. Crowe

Experience has shown that when strict selection for productivity is practised in relatively poor stock, a rapid advance is made for this factor. Unfortunately, once a certain level of production is reached, further improvement is slight; in fact, there is often a back slide.

It is possible that some guinea-pig breeders have met similar setbacks and it may be of interest to examine the methods employed by some of the large-scale breeders of other species.

(A) Hybrids

Outstanding results have been achieved by crossing inbred lines; the resultant F_1 crosses not only surpass the performance of each parent, but are at the same time remarkably uniform in other respects.

Methods of selecting animals to produce these F_1 crosses are as follows.

i. Family Selection. The entire family has to be good and then only the best are chosen from it. Outstanding individuals from a poor family are not used.

ii. Inbreeding within the Family. Full sib matings (that is brother by sister) are practised. This intensifies all factors, good and bad. It is a speedy method of showing up what is in the stock which normal matings may mask. Unless strict selection has been carried out previously for several years, the wastage at this stage will be high.

iii. Progeny Testing. Males which have got as far as this point are now mated to a number of unrelated females. The worth of the male is then assessed on the performance of his daughters.

iv. Crossing outstanding Families. The outstanding proved inbred males of one family are then mated to similarly proved inbred females from another family.

It is not suggested that such a method is practical or necessary for the commercial breeder of guinea-pigs, but it does show what a complicated and costly business it is to increase production beyond a certain point. However, parts of the programme may well be adapted with advantage by breeders with the necessary facilities.

(B) Selection of Boars

The greatest care must obviously be taken when selecting stock boars in view of the influence they have on the entire herd. Instead of picking the best boar out of a litter of five or six and selling the remainder at 10 oz it would seem advisable to rear all the young from the litter to maturity. This would establish whether they are constitutionally sound as a family.

Time and space would probably not permit the testing of the boar's daughters although this would naturally be desirable. However, as a compromise it would be advisable to mate up the sisters to an unrelated boar in order to assess their productivity. It is appreciated that first litters are often smaller, but litters of four are usually possible with good stock.

(C) Selection of Sows

Few breeders use family selection as a means of improving productivity in a herd. All sows from outstanding litters are kept, but those which fail to produce a second litter of at least three are rejected.

The weakness of this method lies in the fact that only the sow which does badly is rejected—the sisters are probably retained. The remainder of the family, even if one has been proved poor, should be removed to ensure they are not used to breed future replacements.

Fortunately there is a clearly defined limit to the number of healthy young a sow can produce in a year under ideal conditions of housing, feeding and management, so the ultimate target is there for all to see. But it must always be remembered when striving after high productivity that laboratories are only interested in receiving healthy animals uniform in weight and size for age. These qualities must not be allowed to deteriorate.

(D) Dangers of Inbreeding

Let us assume a breeder has, after several years of selection within a closed herd, succeeded in raising productivity to a satisfactory level. The next problem is to avoid too high a degree of inbreeding which would quickly undo all the improvement due to selection. Unless full records are kept there is a great risk that this will happen sooner or later.

Small litters, high mortality and general unthriftiness are the usual signs of close inbreeding. Some stock are able, of course, to resist the

effects of inbreeding better than others. But inbreeding, if it is going to be used as one of the tools of the breeder, must be planned. The danger lies in haphazard inbreeding which, because proper records are lacking, goes undetected until too late.

Inbreeding can easily be avoided, of course, by buying in a batch of new males each year. This idea will particularly appeal to the breeder who has a small herd, is limited for space and has little inclination for paper work. Providing the animals are obtained from a recommended source and are isolated for two months, the chances of introducing disease are slight.

It is obvious, however, that if a breeder had, by means of careful selection over a number of years, succeeded in producing a uniformly healthy herd with high productivity it would be a short-sighted policy that risked undoing this work by introducing a boar of unknown quality.

(E) The Closed Herd

The problem facing the breeder is two-fold. Not only will he probably think it desirable to maintain the characteristics of the herd—which necessitates a degree of inbreeding—but at the same time he will wish to devise a simple method by which he can avoid close matings.

Breeders may be interested in having details of a system which can be used with a small herd or adapted to suit the needs of the larger herd.

For the purpose of illustration let us assume a breeder has sixty sows of various ages and a number of boars, all selected for uniformity of production and constitutionally sound. It is not intended in this article to advocate any particular method of management, but the system can be explained more easily if post-partum mating is assumed.

The first thing the breeder has to do is divide his sows into four groups as near as he can judge according to age. To each pen of fifteen sows allocate a boar which is not only unrelated to the sows but is also unrelated to the other boars used. The relationship of the sows is of no great importance, but generally speaking the wider the better.

On the assumption that the useful breeding life of a sow, under intensive methods of management, is not more than 2 years, one of the four pens of sows must be replaced with young stock every 6 months. The pens should therefore be numbered according to age—No. 1 will be the first pen due for replacement.

The basic principle to keep in mind is that when the old sows are removed from pen 1, they are replaced by their own daughters, which

are placed in the actual pen vacated. It will be noted that as there are only fifteen sows in the pen it will take approximately 2 months to select this number of young sows, particularly as only the best are used.

Only the outline of the system has been given. Practical details such as placing discarded sows and young in a spare pen in order that they can have their final litter, arranging pens for rearing replacements, marking young by means of dye or ear punch, etc., can easily be worked out by the experienced breeder.

This system ensures that matings can never be closer than $\frac{1}{4} \times \frac{1}{4}$ (i.e. second cousins). It is essential, however, that the programme is commenced with really first-class stock and that only the best of the young are selected as breeders.

As no records need to be kept and little more is needed than to look at the mating chart once in every 6 months, the system will particularly appeal, it is hoped, to the small breeder who is pressed for space and time.

The basic principle, as already mentioned, can readily be adapted to cover larger herds and with little difficulty can be altered to suit a longer or shorter breeding period according to the individual breeder's preference. In my own case I use a variation of the system to cover a herd of 350 sows which are limited to a breeding life of 18 months before being replaced. They are divided between two buildings and are operated as separate colonies.

6. KEEPING GUINEA-PIGS ON WIRE-MESH FLOORS

G. Porter

There are many types of guinea-pig cages, all of which have been designed for a specific purpose, either to hold experimental animals, for growing on young stock, or as breeding cages.

The design of such cages differs considerably even within any specific group, and while each design has many features worthy of recommendation, not one of them meets with universal approval.

Laboratories and breeders do, however, agree on one very important point; a healthy animal is absolutely necessary for scientific research, and therefore only healthy animals can be maintained by the breeders. From an economic point of view, the cost of producing a healthy animal is less than that for producing an unhealthy one, yet the value of a healthy animal to the laboratory is many times that of a poor unhealthy specimen.

Laboratories will only buy really first-class animals, and if the accredited breeder cannot produce these, large-scale breeding in

laboratories will be considered, although, due to wastage through overproduction, it will be more expensive.

(A) Infections

Respiratory and intestinal infections among guinea-pigs are most common, perhaps due to their unusually high susceptibility. These conditions may be aggravated or in many instances provoked by bad husbandry, e.g. sudden exposure to extremes in temperature, draughty buildings, damp or wet bedding, soiled cage floors, overcrowding, food and water contamination by faecal matter, and inadequate feeding.

(B) Advantages of Wire Floors

To help in the control of these infections a wire-mesh floor is a decided advantage. It eliminates wet and soiled bedding and food, and the animals are prevented from having access to their own and their cage mates' faeces, which must always be regarded as a source of infection. The animals are always dry and clean, all urine and faeces fall through the mesh into the droppings tray. The wire grids can be easily removed, scrubbed or sterilized, dried in a few minutes and returned. There is no possibility of dirty, wet, germ-infested corners being overlooked from one cleaning-out day to the next. There is also a saving on bedding materials. This method may not meet with general approval, but where it can be adopted the health of the colony will undoubtedly improve.

(C) Precautions to be Observed

Guinea-pigs, being rather timid creatures, are inclined to stampede when suddenly startled. It is therefore important, especially where wire-mesh floors are in use, to ensure that no sudden undue disturbance occurs in the animal rooms.

When guinea-pigs are transferred from solid floors to wire-mesh floors, they are inclined to panic when disturbed. This may result in animals getting their legs caught in the mesh. It is therefore advisable to cover the wire mesh for a few days after transfer with a light covering of bedding.

Size of cage or pen is also important. Very large pens are inadvisable, because in such pens the animals chase around, and this again leads to injury. The floor area of breeding pens with wire-mesh floors should not exceed 15 sq. ft.

Size of mesh is of the utmost importance. If the mesh is not the right size, the animals, especially the young ones, are liable to get their legs caught. Experiments have been carried out on different sizes of mesh and it has been found that the most suitable size is 3 in. × ½ in. It has also been suggested that the strands of wire should all be on one level and not interwoven.

(D) Labour Saving

A wire-mesh floor may be suspended over a droppings tray, or as in some modern batteries over removable bitumen-lined paper; either method will greatly reduce the amount of labour expended on cleaning out the animals. If trays are used, they can be scrubbed and disinfected then partly filled with sawdust and returned. There is no danger from traces of disinfectant or from sawdust as the animal cannot gain access to the tray.

It must be remembered, however, that the wire-mesh floors have to be changed, scrubbed or sterilized regularly.

(E) Temperature and Ventilation

The temperature must be kept in the region of 65°F, otherwise the animals will get chilled from sitting on the cold wire; also it is of the utmost importance for these units to be in an absolutely draught-free building.

(F) Summary

With wire-mesh floors the animals are not disturbed by cleaning out. There is less need to handle all animals, and this is especially advantageous for pregnant sows. If a water-bottle leaks it does not flood the pen or cage. Other points of advantage mentioned are control of infection and saving of labour and bedding.

7. COMMON DISEASES IN GUINEA-PIGS[1]

(A) Pseudotuberculosis

Transmission generally by ingestion; mothers may also infect the young. The infection may be either acute, with death occurring within a few days, or chronic, in which case the whole stock may be infected before detection.

i. Visible symptoms. Staring coat, wasting, diarrhoea, rapid breathing and coughing.

[1] Compiled from many sources.

ii. Causative organism. Pasteurella pseudotuberculosis.

iii. Post mortem. Examination generally reveals enlarged, abscessed, mesenteric glands, nodulous spleen and small intestines. The lungs, pleura and pericardium may be inflamed and also contain nodules. Pus may be found in the peritoneal cavity.

(B) Cervical Adenitis

Chronic abscesses in the cervical lymphatic glands. Common in certain colonies; appears to have little effect on health.

i. Causative organism. Group C *Streptococcus*, *Streptobacillus moniliformis* (*Actinomyces muris*).

ii. Post mortem. Examination generally reveals pneumonia or inflammatory changes in the lungs.

(C) Respiratory Tract Infections

Guinea-pigs are susceptible to pneumonia and pleurisy.

i. Causative organisms. Pneumococcus, *Klebsiella*, *Pneumobacillus*, haemolytic *Streptococcus* and *Pasteurella.*

ii. Visible symptoms. Poor condition, staring coat, coughing, sneezing and rapid breathing.

iii. Post mortem. Examination generally reveals pneumonia or inflammatory changes in the lungs.

(D) Intestinal Infections

1. salmonellosis

Most common in the intestinal infections, often reaching epidemic proportions. Diet may precipitate the disease (e.g. frosted or mouldy greens, foodstuffs infected by wild rodents).

i. Causative organisms. Salmonella group, e.g. *Salm. typhimurium*, *Salm. enteritidis.*

ii. Visible symptoms. Staring coat, arched back, wasting and diarrhoea.

iii. Post mortem. Examination generally reveals congested and ulcerated intestines, enlarged spleen, abscesses on the liver, enlarged and oedematous mesenteric lymph glands, local or general peritonitis, possibly pus in the gall bladder.

2. coccidiosis

Very common in the guinea-pig.

i. Causative organism. Eimeria caviae.

ii. Visible symptoms. General loss of condition and diarrhoea.

iii. Post mortem. Examination generally reveals infected gut, tissue damage, nodules on the liver and intestines.

(E) OEDEMA DISEASE

Generally connected with diet, which might contain a toxic substance or else be wrongly formulated.

i. Visible symptoms. Excessive thirst, general loss of condition, obvious oedema in the peritoneum of young animals.

ii. Post mortem. Examination generally reveals gelatineous oedema in the peritoneum, and in more chronic cases liver damage (in adult animals).

(F) VIRUS DISEASES

Apparently healthy animals may carry latent virus infections which could cause confusion when the animals are used experimentally for the study of other viruses.

(G) GUINEA-PIG PARALYSIS

Uncommon virus disease resembling poliomyelitis in man.

Visible symptoms. High temperature, loss of weight, gradually increasing muscular weakness, particularly the hinder parts of the body, paralysis of the hind legs.

(H) SALIVARY VIRUS

Unimportant.

(I) ECTOPARASITES

See Chapter 8.

(J) VITAMIN DEFICIENCIES

Scurvy, or Vitamin C deficiency, common in guinea-pig colonies. An adequate supply of fresh, palatable green food will prevent this condition from arising.

Visible symptoms. Soreness and stiffness of the joints. Swelling and bleeding of the gums, with loosening of the teeth, haemorrhages under the skin.

If scurvy continues untreated, the teeth and bones may become permanently damaged and the animals resistance to infection will be undermined.

2

Mice

1. A PLAN FOR MOUSE BREEDING

F. H. Evans

After many years of experimenting with different methods of housing, feeding and breeding mice, the one described below has proved, in the writer's experience, to be the most efficient for producing uniform mice of the required weight in the minimum time.

(A) Housing

The ideal home for mice has proved to be a wooden box of uniform size which can readily be taken apart for cleaning. Wooden boxes avoid condensation of moisture, but when a box is made entirely of wood the mice will soon gnaw their way out, either through the sides or especially at the corners. To prevent this, slip-out sheets of aluminium (24 gauge) are used to cover the sides of the box, while still allowing a good total area of uncovered wood. Figure 2 illustrates the component parts of this type of mouse box, with their dimensions.

In the lid of the box is an open area 6 in. by 4 in. which serves for ventilation, and a small hole for the water bottle, so placed that it avoids the nesting tray beneath. Inside the lid is a lining of perforated aluminium sheeting, having 45 holes per square inch. The piece of wood removed from the lid so as to make the 6 in. by 4 in. ventilation space serves as a nesting tray. To one side of it is nailed an edge of wood which prevents the litters from falling out, keeps the animals warm and dry and gives them something to chew on, thus sparing the box itself. To replace the tray is, of course, simpler and cheaper than having to replace the box.

The bottom of the tray is covered with sawdust and small wood-shavings. As a lining for the nesting tray, newspaper strips are excellent, being preferred to hay, which is liable to introduce and harbour insects.

(B) Cleaning

The boxes are cleaned at least twice a week and the old bedding replaced throughout by new.

(C) FEEDING

The mice are fed on cubes supplied from a wire-mesh basket clipped on to the side of the box opposite the nesting tray. The food is therefore *inside* the aluminium lining described above.

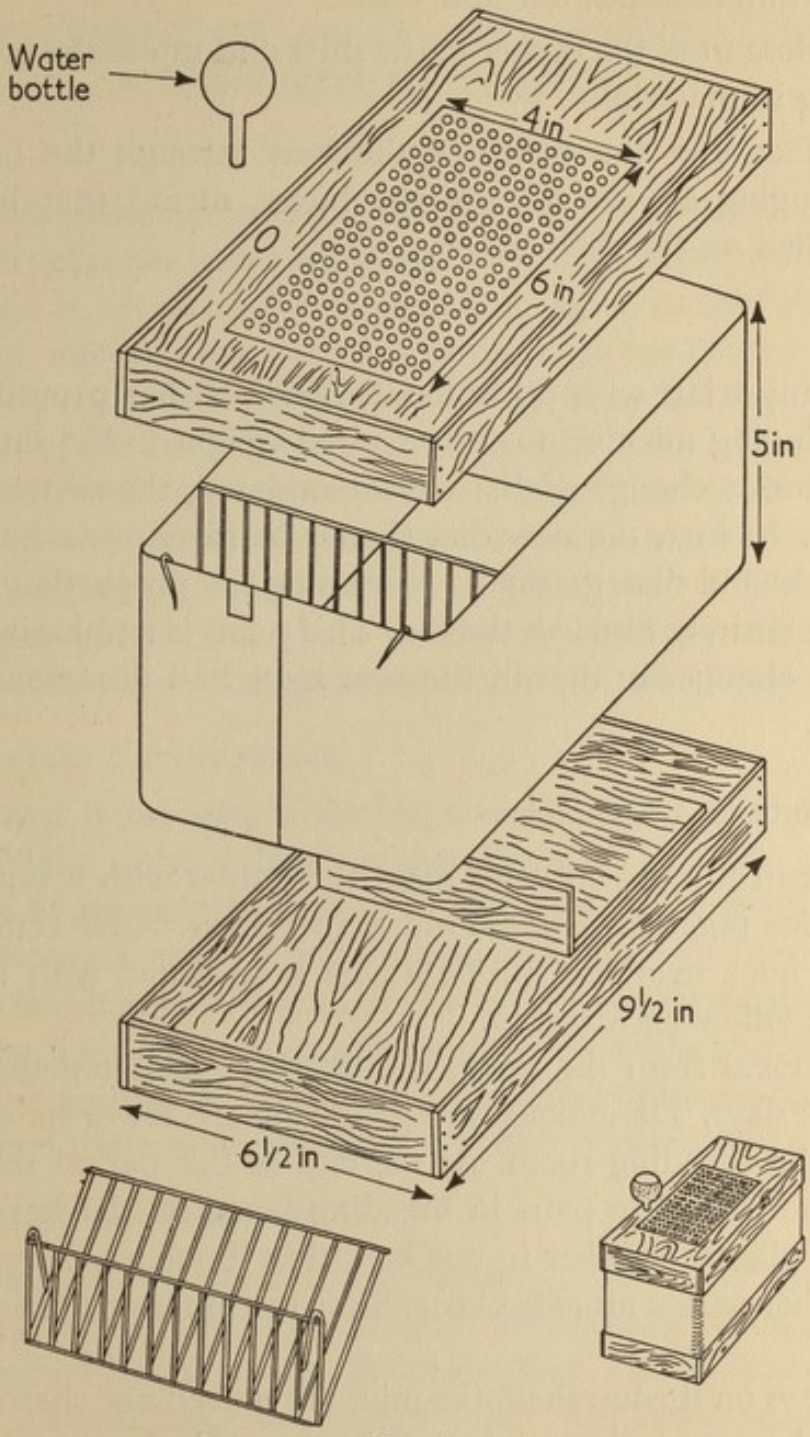


Fig. 2.

The advantages offered by this method of presenting the diet are as follows.

1. Should wild mice enter the breeding-room, they cannot foul the diet as they can if a food trough hangs *outside* the cage.

2. The cubes cannot be contaminated by urine, as often happens when they are scattered on the floor of the cage, instead of being in a suspended container.
3. Cubes remain in one piece until completely consumed, and so do not crumble to powder and waste.
4. The close fit of the basket to the sides and top of the box affords a saving in material.
5. The fact that the water bottles pass through the lids, instead of hanging on the sides of the boxes, means that less room is occupied on the shelves.

(D) Food

After many trials with other foods, Diet 41B has proved to be the best for bringing mice up to the required weight in the shortest time. If at any time a change of diet is contemplated, the switch-over must be gradual. At first, the new diet should be given on a half and half basis with the old diet, gradually increasing the proportion of the new diet until it entirely replaces the old. This point is emphasized because too sudden changes in diet in the past have had disastrous results in this colony.

(E) Breeding

The plan in this colony is to mate three does with a buck, leaving the four mice together from Monday to Friday. After removing and resting the buck over the week-end, he is re-mated with three more does on the following Monday.

After removal from the buck, the does are kept together in large boxes for 14 days. These boxes are placed on the lower six of the eight shelves in the breeding room. At the end of this period the mice are removed and placed in pairs in breeding boxes on the top shelf. Two females are placed together in one box, thus if one mouse proves to be a poor mother or has an extra large litter the other one assists as foster mother.

After 7 days on the top shelf, the mice are moved one shelf down each week until they reach the weight suitable for their despatch to the user. Thus the top shelf becomes vacant as required for the new batch of expectant mothers.

In the selection of breeding stock, the best mice from the largest litters are retained.

(F) RECORDS

The only record kept is of the number of does which fail to become pregnant after mating. These are very few.

2. THE EFFECT OF AN ENVIRONMENTAL CHANGE ON BREEDING MICE

Vivienne M. Balla

Very little information has been prepared and made available on this subject, yet it has been considered by many workers that a constant environment is necessary for laboratory animal production. As with most species, mice are exceptionally sensitive to any change of environment, and do not possess great adaptability, therefore our observations may be part of the answer to the problem of uniform animals behaving differently in various environments. In December 1950 ten pairs of breeding mice were received at the Rowett Research Institute, Aberdeen, from the Institute of Animal Genetics, Edinburgh. The mice were a hybrid strain, and had been bred for eleven generations in one environment, and they were rearing their third, fourth and fifth litters.

(A) EDINBURGH ENVIRONMENT

The animal house was of timber construction with adequate light and ventilation, central heating was installed and the temperature maintained at approximately 60°F. The cages measured 8 in. by 10 in. by 5 in. and were made of solid galvanized iron with perforated lids: a partition was inserted across one end to form a sleeping-compartment or nest. The floor of the cage was covered with sawdust, and hay was provided for nesting. Under these conditions the mice were in dark self-contained units, and could not see their immediate surroundings.

(B) ABERDEEN ENVIRONMENT

The animal house was a brick building with cavity walls; double-glazed windows were fitted to conserve heat. A temperature of 72 ± 2°F was maintained by an air-conditioning plant, and by thermostatically controlled electric heaters. A time-switch was fitted to the artificial lighting system, ensuring periods of 12 hours light and 12 hours darkness daily throughout the year. The cages were made of $\frac{1}{4}$ in. wire mesh measuring 12 in. by 5 in. by 5 in. and fitted into metal trays; this allowed the faecal matter to drop on to the trays, which were filled with sawdust. Sterilized woodwool was placed in the cages for bedding.

Under these conditions the mice were now allowed much more light, and they could see their immediate surroundings, including people working in the room, also other mice in neighbouring cages. The sudden change made them slightly nervous and inclined to bite when being handled.

(C) Feeding in Edinburgh

The mice were fed on Thomson cube (Diet No. 1) of Howie and Porter (1950).

(D) Feeding in Aberdeen

Two diets were tested, twelve pairs being given the Thomson cube as fed in Edinburgh and twelve pairs Diet 86 Howie (1951). Drinking water was given in each case, as these diets are dry and in cube form. The compositions of the diets used are given in Table I.

Table I

Diet No. 1	%	*Diet No.* 86	%
Wheat Offal (Bran)	17·7	Wheat, whole ground	50
Wheat, ground	17·7	Barley, whole ground	25
Oats, Sussex ground	17·7	White fish meal	7
Barley, ground	8·8	Meat-and-bone meal	6
Maize ground	8·8	Dried brewer's yeast	5
Meat-and-bone meal	8·8	Dried grass meal	5
White fish meal	4·5	Cod liver oil	1
Dried skimmed milk	14·0	Salt	1
Dried yeast	1·2		
Cod liver oil	0·4		
Salt	0·4		
	100·0		100

(E) Breeding Procedure

Monogamous mating was adopted, that is each pair remaining together throughout their breeding life; it was noted that the male greatly assisted in keeping the litter warm and hidden from view. The young were weaned when 21 days old, weighed and segregated. Mice for future generations were retained until they were 6 weeks old, when they were mated. This may be regarded as rather young for some strains, but we experienced no difficulties, and this had been the Edinburgh procedure.

(F) OBSERVATIONS

Birth dates, the numbers born alive or dead, the numbers weaned and the body weights of males and females at 21 days were recorded.

(G) RESULTS

In 1951 the number of animals weaned from the first litters was very small on both diets, as many of the young were eaten during the first 3 days of life. This was attributed to the nervous disposition of the animals in new surroundings, and the lack of privacy in their cages. The percentage weaned from subsequent litters increased markedly, as the animals became adapted to the new environment. By January 1953 this strain of mouse was entirely acclimatized to the Aberdeen environment and, as a point of interest, a further breeding test was designed to compare this breeding performance with that of the first generation reared in Aberdeen. Twenty-four pairs of 6-week-old mice were mated, twelve pairs on each of the two diets. The conditions were identical with those of the 1951 test, including the season of the year; Table II gives the details of both tests. Incidental deaths in the breeding stock slightly reduced the number of does as shown in the last column of this table.

The mice tested on Diet 86 were transferred from Diet 1 at mating, and in familiar surroundings the change of diet proved no disadvantage. While the birth rate was not significantly different, the percentage

TABLE II

			Av. No. per Dam			*Av. Weaning Wt.*		*No. of Dams in Gp.*
Year	*Litter*	*Diet*	*Born*	*Weaned*	*% Weaned*	♂	♀	
1951	1st	1	8·9	3·1	34·7	8·1	8·6	11
	,,	86	7·8	2·0	25·5	8·7	9·3	12
1953	,,	1	7·7	6·6	85·9	10·6	10·8	12
	,,	86	8·6	7·0	81·1	10·2	10·3	11
1951	2nd	1	10·0	8·7	87·3	9·6	8·5	11
	,,	86	7·5	4·8	63·3	9·4	9·4	12
1953	,,	1	8·2	7·1	87·1	11·3	10·9	12
	,,	86	10·0	8·8	88·2	11·0	9·9	11
1951	3rd	1	9·2	6·2	71·8	9·8	10·3	10
	,,	86	8·3	6·6	79·8	10·1	9·7	12
1953	,,	1	8·1	7·6	93·9	11·2	11·7	12
	,,	86	10·2	8·6	92·2	11·2	11·0	11

weaned and body weights were considerably higher in 1953, especially for the first litters. Weaning weights were greater when the percentages weaned were greater, which is rather surprising and emphasizes the fact that mice not accustomed to disturbances such as were present in the Aberdeen animal house do not lactate well.

(H) Summary

As a result of our observations it is suggested that environmental change is a variable which at all times should be avoided if maximum productivity is to be obtained. Any differences observed between diets are probably related to environmental factors.

References

Howie, J. W. (1951). *J. Anim. Tech. Ass.* **2**, 7.
Howie, J. W. and Porter, G. (1950). *Brit. J. Nutr.* **4**, 175.

3. REFLECTIONS ON THE COMMERCIAL PRODUCTION OF LABORATORY MICE

A. A. Tuffery

Commercial breeders supply about half of all the mice used in the United Kingdom, and will probably never supply a much bigger proportion. It seems to me that there are two basic reasons for this state of affairs, namely, inability to supply particular types of animals and the problem of quality. First, we may briefly examine the question of type of animal. Workers using mice for the study of problems in cancer and genetics need to use inbred animals, of an absolutely guaranteed pedigree, and the organization of the average commercial colony is not geared to cope with this type of breeding. Further, it is doubtful whether the commercial breeder has the basic scientific knowledge needed in some aspects of this special type of breeding. Again, other mouse users, especially in the field of virology, require very young—new-born or 2-3 day old—animals, and it will be quite obvious that these must of necessity be produced just where they are to be used. Here then, are two cases where the average accredited breeder is unable to supply a laboratory's needs.

Before the question of quality is dealt with, mention can be made of some very distinct advantages the commercial breeder has. In

general (though I doubt whether always) his mice are cheaper than comparable laboratory-produced animals, and this would appear to be due partly to the fact that large-scale production will always be the more economical. If he is working on a very large scale he can also accept, without too much dislocation in his colony, sudden large orders for mice—an occurrence not unknown in the world of laboratory users. This, however, does apply to only a very small proportion of breeders, perhaps three or four, and the smaller concern will be far happier to deal with a more restricted but steady demand from just a few customers. Finally, some laboratories, especially small-scale or irregular users, will be very much relieved if they have not the responsibility of producing their animals—frequently they have neither the labour nor the room to breed mice.

So long as a laboratory is satisfied with the number and quality of animals, it will continue to do business with a regular supplier, but should it find that, because of the work being carried out, a higher grade mouse is needed than the one it has been using, one of two alternatives has to be faced by the breeder. Either he loses the custom or he must do something about the quality of his animals.

There was a time in the history of the use of animals in biological work when any animal would do provided that it could be reasonably expected to survive long enough for the experiment in hand. Those days are well past, and laboratory animals are of a far higher quality than before the war. Nevertheless they can still be improved upon. The requirements of all animal users are becoming, and will become, more and more exacting, and if the commercial breeder is to maintain his prominent and, at present, valuable position, he must be prepared to cater for these higher demands. There are breeders who are spoken of very highly and who concern themselves with the needs of their customers. Problems of animal health and diseases are not the only ones involved in the general term "quality", but they are extremely important.

With regard to disease, however, it is not just enough that the breeder himself sees no untoward deaths in his colony. Infections caused by bacteria, viruses, worms and so on can only too often be present without showing any outward signs for long periods. It should be of very great concern to the breeder how his animals behave upon receipt at the laboratory, and whether they die of, say, ectromelia during use. If mice show any signs of disease within a few days of arrival at their destination, then that disease must have travelled with

them from the producer's colony or have been contracted in the laboratory's animal house. Unfortunately, it must be admitted that the laboratories are sometimes to blame, but not nearly so often as some breeders like to think. This is so only in a small proportion of cases, and then it is for the laboratory to take action. But the breeder does sometimes hear reports of his mice not being up to standard and, where this is so, particularly if it is a question of disease, he must take steps to remedy the trouble.

The agents of disease—the bacteria and viruses—can exist in a mouse colony more or less permanently without that colony giving any signs of their presence; there is no epidemic resulting in a sudden increase in the daily death rate, for example, and herein lies the danger. When mice from such an infected colony are sent to a laboratory in apparent good health, the resulting environmental changes to which they are subjected (different cages, bedding, food, temperature, etc.) and experimental stresses made upon them enable the infecting agent to take the upper hand—the disease flares up and kills the mice, much to the breeder's surprise and the laboratory's annoyance. In the breeding colony one has to combat the spread of these disease-producing agents at all times, whether one is experiencing a bad outbreak or not.

Food can be an extremely efficient means of both introducing and aiding the spread of infection. In the first place, give a great deal of thought to its storage—it must be stored well away from the colony, and must not be accessible to wild mice and rats. A number of dustbins with tight-fitting lids will be very useful. And then—why use a mash? It is very messy and takes a lot of work to prepare, and when dropped on to the floor of the cage, as it usually is, is a quick way of spreading infection. Mouse typhoid, like human typhoid fever, is spread by food and water which have been contaminated by infective faeces. The readiness with which a mash diet is fouled is obvious. Cubed diets, on the other hand, take no preparation and, when held in some kind of hopper, are virtually uncontaminated by the mice. One does, of course, need to use water bottles if cubes are given, but these do not give much additional labour. Much more is expended on mixing a mash. There is no question of the advantages of cubes from the hygienic point of view, and ultimately questions of hygiene will force one to take action. Almost every laboratory has long since given up using mash-type feedstuffs in favour of cubes.

Cages, kept on racks for ease of handling and inspection, must be adequately cleaned at regular intervals, and bedding must be clean

and fresh. Hay is a popular bedding material among commercial colonies, but few laboratories favour it—woodwool is far less likely to act as a vehicle for infection. Hay is not needed for the mice to eat, but if it is used as a bedding in unheated or poorly heated animal quarters then its use may be justified. Take care over its storage and try to buy it from sources where it is unlikely to have been contaminated by wild rodents. The same remarks apply to sawdust and woodchips if these are used. Sawdust particularly is liable to be left lying unprotected in open yards, and is very readily fouled by rats, mice and cats (cats carry a tapeworm which can infect mice, encysting in the liver). Some breeders have their supply of sawdust bagged in their own sacks direct from the saw bench, which seems a very good idea.

Commercial mouse-breeding colonies will also be designed to produce their mice as economically as possible—it is, after all, a business, and yet I am not at all convinced that the systems in general use are the most economical. The standard methods used involve mating several does to one buck; the does are then removed at pregnancy, and are allowed to litter down and nurse their young away from the male. Therefore, mating at the *post-partum* oestrus does not occur. If a system involving *post-partum* mating was used, the usefulness of the doe (i.e. her productivity) would be very much increased. Indeed, one would need to feed a higher proportion of males (using one male to two females), but the useful breeding life of the unit could be shortened, giving one a more rapid turnover in breeding stock.

One very important means of improving and maintaining the quality of one's animals is to practise some kind of selection when the breeding stock is being replaced. It is not sufficient merely to select the better looking animals for this purpose—one must give a little thought to what one wants to do, and how to do it, and in this respect I would urge breeders to re-read the excellent article by Dr Falconer (p. 111), where the principles of the process are fully and simply explained. One requisite for the intelligent practice of selection is some kind of recording system. How can one really tell if one's colony is as good now as it was a year ago if all one has is a ticket with the date and number born pinned to the side of the box?

This short contribution is not primarily concerned with detailed practical advice to breeders, since this can easily be obtained from other articles in this book, but the most important point to be emphasized is this: laboratory demands for mice will, without any doubt, become more exacting. In the first place, mice are an expensive item

to any laboratory, but of far greater importance is the fact that animals used in all the various types of work must give consistently satisfactory results in the tests for which they are used. It may be argued that one customer seems to be quite satisfied since he never makes any comment on the quality of one's mice, but let us hope he does not hold the view that he must make the best of a bad job. Eventually he will have to do something about it—even breed his own mice at twice the cost.

The commercial breeder is doing a valuable job at the present time, and is appreciated as such, but the commercially produced mouse will, unless something happens to its quality, become less and less suitable for the uses to which it will, undoubtedly, be put in the future. It would be more than rash to forecast a day when mouse breeding will cease to be profitable, but there is still time to consider this point and act upon it. If sufficient action is taken, there is no reason for it not to remain a profitable occupation.

4. COMMON DISEASES IN MICE[1]

(A) Intestinal Infections

1. salmonellosis

Very prevalent in mouse colonies, often reaching epidemic proportions, may remain undetected or latent until the animal is put under stress. Animals which recover may become carriers and infect the whole colony.

i. Method of Control. High standard of hygiene. Destroy sick animals and contacts. Ensure foodstuffs are not contaminated by wild species. In an epidemic it may be necessary to destroy whole stock. For other information see page 35.

(B) Septicaemic Diseases

These diseases are generally acute and fall into three types, namely Pasteurellosis, Pseudotuberculosis and Mouse Septicaemia. Sudden death gives insufficient time for characteristic morbid changes to take place. Post-mortem examination provides little evidence of the disease.

1. pasteurellosis

Very rare. Belongs to the so-called haemorrhagic septicaemia group.

i. Causative organisms. Pasteurella muriseptica (*Pasteurella muricida*).

[1] Compiled from many sources.

2. PSEUDOTUBERCULOSIS

Several types, one of which may be connected with a *Salmonella* organism; another variety which may reach epidemic proportions is caused by *Corynebacterium pseudotuberculosis*.

3. MOUSE SEPTICAEMIA

May be caused by *Erysipelothrix muriseptica*, which is allied to the *Erysipelothrix rhusiopathiae* organism of swine erysipelas.

(C) STREPTOBACILLUS MONILIFORMIS INFECTION

There are two types of infection, namely acute and chronic. Animals suffering from the chronic type may recover and acquire an immunity, but will continue to spread the infection because they may be carriers.

1. ACUTE STREPTOBACILLUS INFECTION

i. Visible symptoms. Staring coat, conjunctivitis, eyelids glued together. Death within a few days.

ii. Causative organisms. Streptobacillus moniliformis (*Actinomyces muris*).

iii. Post mortem. Examination may reveal no characteristic changes.

2. CHRONIC STREPTOBACILLUS INFECTION

i. Visible symptoms. Staring coat, emaciation, conjunctivitis. Oedema of extremities, ulceration of feet, paralysis of hind legs.

ii. Causative organisms. As above.

iii. Post mortem. Joints contain thick caseous exudate (advanced cases only), enlargement of lymph glands, abscesses in the spleen, pericarditis.

(D) INFECTIOUS CATARRH

Frequently referred to as mouse catarrh. The disease may be sporadic, in which case the infected animals should be killed and cage contacts isolated for observation. In other cases it may be epidemic, when all animals showing any symptoms and all contacts should be killed. Maintain a high standard of hygiene.

i. Visible symptoms. Intermittent chattering, wheezing, rhinitis, respiratory distress, staring coat, conjunctivitis.

ii. Causative organisms. Coccobacillary bodies regularly found in exudates. Possibly related to P.P.L.O. (Pleuropneumonia-like organisms).

iii. Post mortem. Examination generally reveals rhinitis, inflammation of the internal or middle ear. Pneumonia.

(E) Pyogenic Infections

Not very common. Occasionally epizootic due to haemolytic streptococci.

i. Visible symptoms. Animal looks ill, staring coat, rapid breathing, subcutaneous abscesses about the head and neck.

ii. Causative organisms. Staphylococcus aureus.

iii. Post mortem. Spleen may be enlarged, with pin-point abscesses.

(F) Focal Necrosis of the Liver

This is quite common and may be widespread throughout some colonies. May be sporadic or epidemic. First described by Tyzzer in a colony of Japanese waltzing mice. Only symptoms before death may be slight diarrhœa. Often flares up when animals are under stress. Cause of this disease debatable, but it may be associated with *Bacillus piliformis.*

i. Post mortem. Examination reveals enlarged liver, containing multiple yellow necrotic lesions, with dark central areas.

(G) Mouse Pox or Ectromelia

Common disease due to a filterable virus. May remain latent until animals are under stress, when it becomes epidemic.

i. Visible symptoms. (Acute type) Death may occur in a few hours, without visible symptoms. (Chronic type) Staring coat, general condition poor, enlargement of one foot (usually a hind one), bulbous swellings on the tail. In the advanced stage, exudation of serous fluid and scab formation on foot, swelling of whole leg, gangrene of a toe or whole foot.

ii. Post mortem. Examination generally reveals generalized congestion, small haemorrhages, excess fluid in the peritoneal cavity, diffuse necrosis in the liver and spleen.

iii. Control. In the absence of expert advice, an infected colony should be destroyed. Cages must be sterilized, racks, walls and utensils chemically disinfected and room allowed to remain vacant for some time. Vaccination can be carried out and, if correctly done, may successfully control an outbreak.

(H) Virus Diseases (Other than ectromelia)

1. lymphocyctic choriomeningitis

Not very common, often latent, but infective to humans.

i. Visible symptoms. Loss of weight, hunched back, conjunctivitis, spastic convulsions or partial paralysis affecting hind legs.

2. ENCEPHALOMYELITIS

Uncommon, often latent until animal is under stress. Death within a few days.

i. Visible symptoms. Loss of weight, hunched back, conjunctivitis, spastic convulsions or partial paralysis affecting hind legs.

3. VIRUS PNEUMONIA

Not uncommon; several types encountered. May be latent, until animal is under stress.

i. Visible symptoms. Staring coat, poor condition, rapid breathing, followed by death.

ii. Post mortem. Examination generally reveals pneumonia or inflammatory changes in the lungs.

3
Rabbits

1. THE MANAGEMENT OF A RABBIT COLONY

G. B. Parkinson

The management of a rabbit colony must be approached from two distinct viewpoints; firstly the breeder, who must consider the economics and how he can best serve the laboratories, secondly the research worker, who wants an animal conforming to definite standards of a given breed with a high degree of uniformity. For many years I have been trying to cover both viewpoints in order to benefit myself (the producer) and the research worker (the user).

The methods described here have met with a degree of success, but if there is one major factor of which I am being constantly reminded, it is the necessity to provide for the breeding doe warmth and a feeling of absolute security, as this does, without question, result in increased production and high-quality animals. It also appears to decrease the incidence of scouring, which is, I believe, due mainly to stress factors related to the natural timidity of the animal.

(A) Housing and Equipment

Figure 3 shows a double breeding pen, which can be arranged in lines of one to six, or in blocks of eight. The run walls are made of 1 in. wire netting, the floors of $\frac{1}{2}$ in. by 18-gauge wire netting, or similar heavy gauge wirework, and the hayrack partition is also of 1 in. wire netting. The overall length is 5 ft. 6 in. and the width 1 ft. 6 in. This size is quite adequate to accommodate a doe and eight young until the latter have reached the weaning age of eight weeks, weighing 3-4 lb.

The nest box is 1 ft. 6 in. by 1 ft. 6 in. made of hardboard walls, and $\frac{1}{2}$ in. timber floor and lid. The size is important, because if a nest box is of larger dimensions the doe is inclined to soil the nest. It is possible for the nest box to be smaller, as the doe only stays in the box when suckling her young, but I have not put a smaller nest box into practice.

These rabbit pens are placed under lean-to shelters measuring 48 ft. by 20 ft. I have found the nearly open air situation has resulted in a complete absence of respiratory troubles.

Feeding equipment is quite simple for both breeding and running-on pens. Pellets are fed *ad lib* in feeders to all stock. Water is available at all times.

(B) BREEDING

Over the years I have developed a colony of large albinos and a smaller colony of chinchilla giganta, which I occasionally cross to maintain hybrid vigour. I have also used other breeds, particularly English crossed with albinos, with varying success. Research work demands various types and breeds, but albinos satisfy most requirements.

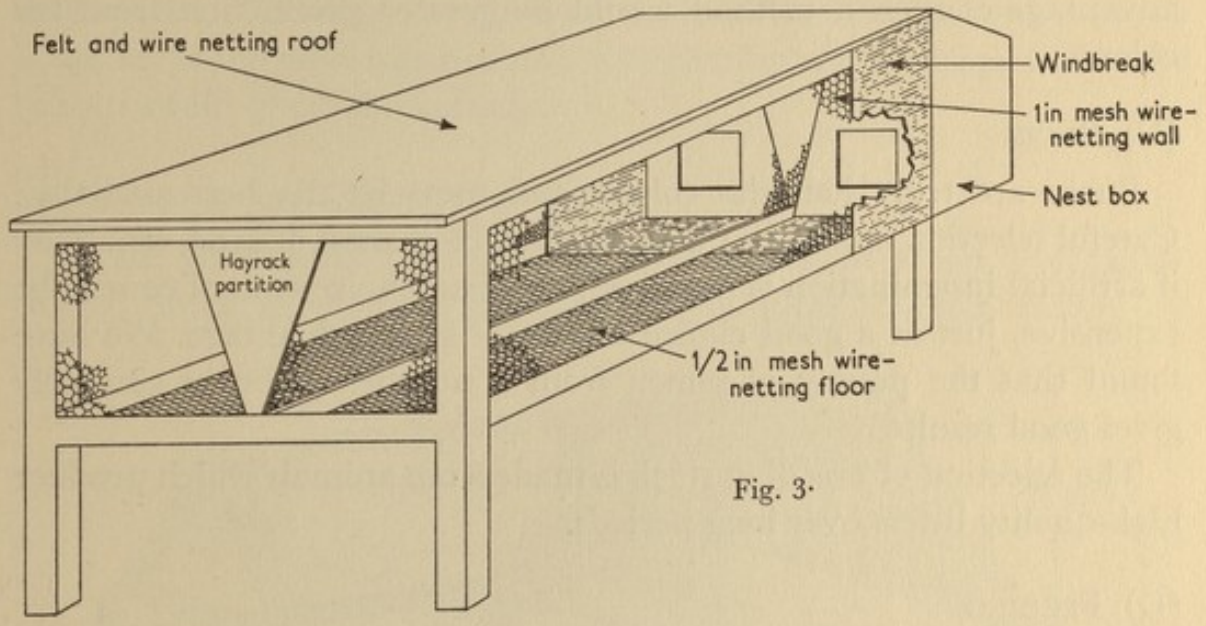


Fig. 3·

Mating is done in batches of six, either by individual matings or running six females with one male. The former method is preferable as the does all kindle at the same time, which enables fostering between litters to be practised. The latter method is useful when one is short of breeding cages, by this method it is also possible to keep a larger number of breeding does.

Artificial insemination has a lot to offer the commercial breeder and will become more widely used in the future. In co-operation with Dr C. E. Adams, I have used this method very successfully, but have not put it into general practice.

A check for pregnancy by palpation is made 14 days after mating. If the animal is not pregnant or in pseudopregnancy, re-mate after the 17th day.

On the day of parturition, the litter is inspected and the number of

young reduced to eight per litter. The surplus of young are either destroyed or fostered on to other does with smaller litters.

The nest is frequently examined during the first few days, when the progress of the young is noted and any dead removed.

Young rabbits are usually weaned at 6 weeks of age and the does re-mated as soon after as possible, this cycle continues without a break. The does are not rested, but they do rest, however, by becoming pseudopregnant, or refusing to mate. In my opinion, rabbits could be weaned at 4 weeks of age if there existed (*a*) a good pellet of perhaps a higher nutritional quality than those normally used, and (*b*) better brooding equipment which would provide very warm conditions. The advantage of such a method would be greater production from the colony.

(C) Bucks

Bucks are truly half the colony and must be the best available. Careful selection should be made for the factors most desired, especially if artificial insemination is practised, as a bad choice can, of course, be expensive, just as a good choice can be of great advantage. We have found that the mixing of semen from a number of bucks generally gives good results.

The selection of breeding stock is made from animals which produce high-quality litters over long periods.

(D) Feeding

There is a lot to be learned about feeding rabbits, and I have done much experimenting without any spectacular success. I tried S.G.1 just because it was new, and since then I have used nothing else. Short and Gamage, who produced this diet, have made a wonderful contribution to rabbit breeding and they have my grateful thanks. All animals are fed *ad lib* and are provided with continuous fresh water. I have cut out hay or any additional food other than barley straw, which is primarily used for bedding, but it is eaten quite readily.

I do not use a coccidiostat in the diet, I prefer to use nitrofurazone in the drinking water for five days immediately after weaning.

In the control of coccidiosis, wire floors are also a decided advantage.

It is true the young when leaving the nest are loth to move on to the wire at first, but a thin layer of straw for a few days quickly overcomes this, and with wire floors, cleaning out is not the filthy, laborious job which it is when rabbits are kept in hutches with solid floors.

Commercial rabbit breeding presents a challenge, consequently a flexible and alert approach to the problem is of the utmost importance if the breeder is to produce a worth-while animal which will satisfy the research worker.

2. RABBIT BREEDING AND REARING ON GRASS

Barclay S. Walker

This trial was designed to test whether or not the domesticated rabbit could be bred and reared on the natural herbage available to the wild species. We adopted a method known as the Morant system which is the use of movable hutches with wire-netting runs through which the rabbits could graze the herbage.

(A) Housing and Equipment

Figure 4 shows the type of hutch used. It consisted of a triangular-shaped hutch to which is attached a light frame covered entirely by

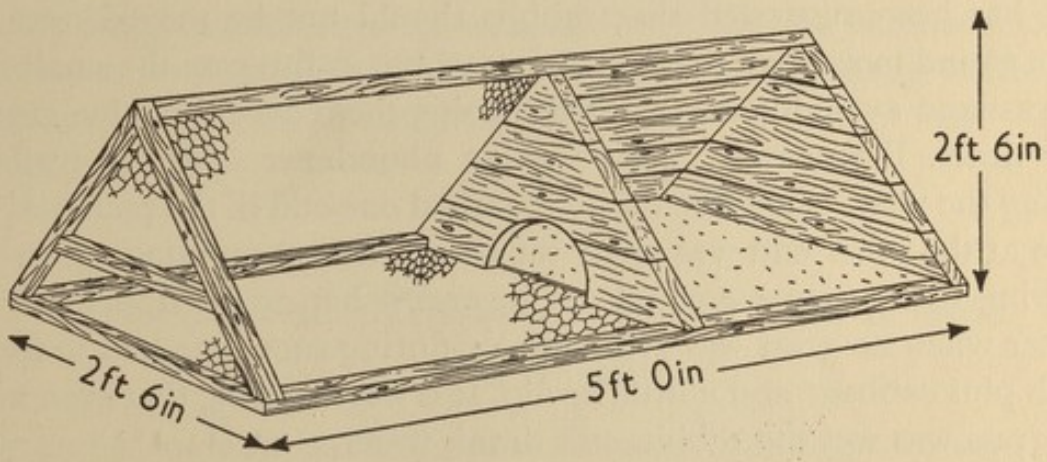


Fig. 4. Rabbit fold (Morant system)

1 in. mesh wire netting. These hutches were constructed of good-quality, well-seasoned wood to withstand the weather, daily moving and the natural enemies of the rabbit. The entire unit was treated periodically, inside and out, with creosote as a preservative and a disinfectant. A zinc roof to the sleeping quarters is more durable than a wooden one, but even in Scotland proved to be too hot in the summer and condensed moisture in the winter. The initial cost of this unit was rather high, which was more than compensated for in reduced feeding costs and saving of labour.

It is interesting to note that the rabbits generally went into the run before defaecating and urinating, and thus kept their sleeping quarters clean and dry. The hutches were cleaned out twice weekly and replenished with oat-straw bedding.

The floors of the sleeping quarters can be fitted with a push-in galvanized iron tray, though we preferred to have several small drainage holes bored in the floor boards. Galvanized metal water pots were clipped on to the side of the run. These pots were placed sufficiently high to prevent the water from becoming soiled by the rabbits.

(B) Feeding

Eight hutches were folded over a half-acre paddock which consisted essentially of perennial ryegrass and clover, with a small quantity of cocksfoot and timothy. The folds were moved four times daily, at 9 a.m., 12 noon, 2 p.m. and 5 p.m., giving the rabbits continuous access to fresh grass, and at any one of these times the water pots could be refilled.

It has been suggested that rabbits should not be moved over the same gound more than twice in one year, but in this case the small area of grassland available necessitated moving them over the same ground four times. Fortunately, there was an abundance of grass available during the year. When the rabbits reached one end of the paddock, the grass at the other end was ready for use, in fact at one stage grass was growing faster than we could use it, and it had to be tipped. In the winter when no grass was available, or during snow, we had to feed a mash plus cabbage and a little turnip. It is worth noting that even when the grass was wet the rabbits still drank water.

(C) Breeding

Before commencing this method of rabbit keeping, a careful selection of the strongest and healthiest rabbits must be made.

Of five young chinchilla does which were mated, four became pregnant, produced and reared twenty-eight young. The fifth rabbit exhibited signs of pseudopregnancy, a comparatively rare occurrence in this strain. This condition was suspected on the 20th day after mating when this animal started pulling out fur and building a nest. On palpation she was found to be empty and was therefore placed with the buck; mating took place and 31 days later a litter of five was born.

The litter was quite normal, coming out into the run on the 12-13th day and nibbling the grass.

All young were weaned at 8 weeks, the sexes segregated and the animals placed in communal hutches, which were made by joining together two of the ordinary breeding hutches. The stock remained in these hutches until they were old enough to be used for experimental work or breeding.

The rabbits thrived well on this system, with an average weight of 6-7 lb. at maturity. There were no deaths from natural causes. In winter, when the rabbits kept in heated quarters were suffering from respiratory infections, this colony was absolutely free from any such complaint, even though the winter was one of the most severe.

These rabbits were fed entirely on grass for 6 months of the year, and in other parts of the country where the winter is less severe, this period could undoubtedly be prolonged, thereby reducing the cost of rabbit production.

This method of rabbit keeping is used extensively on the Continent, but on a much larger scale, with hutches and runs measuring 30 ft. by 12 ft., which can accommodate about twenty rabbits. They are fed entirely on grass and fatten up well, and are therefore in great demand for human consumption. This is also an easy and cheap method of producing rabbits for the laboratory.

3. ARTIFICIAL INSEMINATION IN THE RABBIT

C. E. Adams

In recent months a considerable interest has been shown in the technique of artificial insemination as applied to the rabbit, especially with reference to commercial rabbit breeding. The technique, in itself, is not new, having been used under laboratory conditions for nearly forty years. However, its use has been largely restricted to research problems and there has been very little application to practice. This is surprising in view of the considerable advantages it offers over natural mating, particularly when dealing with large numbers of animals. Amongst these advantages the following may be enumerated.

i. High rate of success. Conception rates (i.e. proportion of does becoming pregnant) approaching 90% can be obtained. In a total of 377 inseminations performed from 1957 to 1960 at the Animal

Research Station, Cambridge, 310 does (83·6%) produced litters. The litter size equals that obtained with natural mating.

ii. Better organization and planning of a breeding programme. Groups of does can be inseminated on a particular day, and all their offspring can be weaned simultaneously, thus "streamlining" a production unit.

iii. Speed of operation. It is possible to inseminate about thirty does per hour. As a hormone injection is used for the induction of ovulation, it is not necessary to observe whether the does will accept the buck.

iv. Selected males. More efficient use can be made of selected males. It is estimated that one buck could provide sufficient semen annually for the insemination of 2,000 to 5,000 does.

v. Disease control, e.g. Syphilis and Snuffles. A review of recent literature, containing approximately one hundred references appertaining to artificial insemination in rodents has recently appeared (Adams, 1962), whilst a comprehensive account of the practical aspects of A.I. is also available (Adams, 1961). For this reason, only a bare outline of the technique will be given on this occasion.

(A) Collection of Semen

Semen is collected from the buck with the aid of an improved type of artificial vagina, as described by Walton (1958). Ejaculate volume usually varies from 0·5 to 1·0 ml while sperm density may vary within very wide limits: a good sample will contain more than 200 million sperm per ml.

(B) Dilution of Semen

Rabbit semen can be diluted with a physiological saline solution, e.g. 0·9% sodium chloride, or Krebs' Ringer bicarbonate, to which a little glucose (0·25%) and penicillin (1,000 units per ml) may be added. The degree of dilution will be governed by the density of the sample. Very little exact information is available regarding the storage of rabbit semen. It is not known for how long the spermatozoa can survive outside the body and retain their capacity to effect fertilization. It is worth noting that the latter function disappears before the spermatozoa lose their power of motility. As a general rule, to be followed until more facts on storage are gathered, semen should be used within 12 hours of collection.

(C) Insemination

A special pipette is used for introducing the spermatozoa into the

female genital tract. The techniques of restraining the doe and of guiding the pipette into the vagina have been described elsewhere. (Adams, 1961). Each doe should receive about 20 million spermatozoa in 0·5 ml of fluid; this number is more than adequate, as it is known that 1 million spermatozoa are sufficient to effect maximal fertilization.

(D) Induction of Ovulation

Ovulation is induced by the intravenous injection of 20 i.u. luteinizing hormone (LH). It has been found that this method causes nearly 100% of does to ovulate, though it suffers from the disadvantage that a state of refractoriness to the injection eventually develops. However, in such animals, ovulation can still be induced by mating with either fertile or vasectomized (sterile) males. Induction of ovulation and insemination need not take place concurrently. In fact, the insemination can be carried out several hours before, or up to 6 hours after, giving the LH injection.

In conclusion, it is worth emphasizing that for A.I. in the rabbit, no expensive equipment is required and a knowledge of how to perform the technique is easily acquired. Whether it will become adopted as a standard technique in rabbit breeding will probably depend upon the growth of larger breeding units in which it must surely compete favourably with the traditional system of natural mating.

References

Adams, C. E. (1961). "Artificial Insemination in the Rabbit." *Tech. Bull. No.* 1, Commercial Rabbit Association.

Adams, C. E. (1962). Artificial Insemination in Rodents *in* "The Semen of Animals and Artificial Insemination", Chap.18. Commonwealth Agricultural Bureaux.

Walton, A. (1958). *J. Physiol.* **143**, 26-28.

4. COMMON DISEASES IN RABBITS

P. L. Shanks

(A) Coccidiosis

Coccidiosis is probably the most common disease of both domesticated and wild rabbits, and until the introduction during the past few years of satisfactory medicines to deal with it, coccidiosis was responsible for more deaths than any other disease.

There are two forms of the disease in rabbits, one affecting the liver and producing white spots to which the term "spotted liver" has been

applied, and the other affecting the intestine. Both forms may be found in the same rabbit although the parasite in each case is different. The coccidia of rabbits will not affect any other animal, neither will the coccidia of any other animal affect the rabbit.

Most adult rabbits harbour the parasite either in their livers or in their intestines, and because they have built up a resistance no illness is noticed. But these adult carriers can, through their droppings, contaminate the food of young rabbits and cause a heavy infestation before time has permitted the youngsters to develop a resistance.

The coccidium multiplies inside the animal body but has to be passed out in the manure before it can "ripen" and again multiply in the body. In theory, therefore, it should be possible to eliminate the disease completely by killing off all the parasites in the liver or intestines and then seeing that the rabbits are kept free from fresh infestations.

The disease is usually seen in young rabbits either while still suckling their dams or more frequently soon after weaning. There may be no particular symptoms apart from the fact that the young rabbits are not growing so well as they should. Death may be sudden, especially following a very heavy infestation. Usually the disease is chronic, the young rabbits having a lustreless fur and being disinclined to play and romp around.

Diagnosis of the disease can be made by having droppings examined microscopically. If deaths have occurred, smears made from the spots in the liver or from the inner lining of the intestine will show large numbers of coccidia. The white spots in the liver could be confused with spots found in cases of pseudotuberculosis, although in that case white or yellowish spots are usually found also in the spleen and intestine.

Treatment with sulphamezathine and a variety of similar drugs has proved very successful. It may be given in the mash or in the drinking water and should be given at the dosage rate recommended by the manufacturers of the drug. For preventive purposes young rabbits may be given the same drug in their drinking water from weaning until 4 or 5 months old.

After an outbreak of coccidiosis hutches and utensils should be disinfected with 10% commercial ammonia. Ordinary antiseptics are not satisfactory because the parasite has a thick "shell" which is resistant to them.

Coccidiosis is no longer the major disease risk of rabbit breeders that it was a few years ago, but it is still capable of flaring up if adequate precautions are not taken.

(B) SNUFFLES

This, as the name implies, is a disease affecting the nose, producing symptoms which are characterized by snuffling. It is a bacterial infection of the respiratory tract. In mild cases, apart from the sneezing and a nasal catarrh, affected rabbits are not really ill. A secondary infection, however, may become superimposed on the mild catarrh, resulting in a purulent discharge from the nose leading to exaggerated snuffling symptoms which may terminate in death from pneumonia. Occasionally a more acute form of the disease occurs when the infection causes a severe fever resulting in septicaemia and death in two or three days, usually from pneumonia.

This disease in the mild form as well as in the acute is very infectious and will spread rapidly through a rabbitry. For that reason any rabbit showing suspicion of snuffles should be removed immediately from its cage and kept in isolation. Its cage mates should also be isolated in case they should be incubating the disease. The cage itself should be thoroughly cleaned out, the bedding destroyed and everything which has been in contact with the affected rabbit thoroughly disinfected.

Treatment using injections of antibiotics is fairly satisfactory in curing all but the very acute cases. Prevention by isolation and disinfection is of prime importance.

(C) RABBIT SYPHILIS

This is a true venereal disease of rabbits caused by a spirochaete which affects rabbits, and rabbits only. In a breeding unit it can be spread rapidly by an infected buck. The disease appears first on the external genitals of either the male or the female as small moist ulcers. These may extend from the original site and involve the inner aspect of the thigh. Occasionally the mucous membrane of the eye, mouth and nose may become affected in cases which have been neglected. In bad cases there may be difficulty in passing pellets and urine.

While not usually a fatal disease, it is very repulsive. Treatment with injections of arsenical preparations is highly efficient. Cleaning and disinfection of infected cages should be carried out to kill off the infection.

When buying rabbits for breeding, a careful examination should be made for evidence of the disease.

(D) BLADDER WORMS

These are the cystic stages of tapeworms which are found in the dog. There are two forms, *Cysticercus pisiformis*, the cystic form of *Taenia*

pisiformis, and *Coenurus serialis*, the cystic form of *Multiceps serialis*. While these cysts are quite common in wild rabbits, they are uncommon under domesticated conditions except when foodstuff, particularly grass, is fed which has been contaminated with dog faeces.

The cyst of *pisiformis* is found in the abdomen often in the region of the liver. The number of cysts varies from one to several. It is rare for them to cause ill-health and they are usually seen only on post-mortem examination. Each cyst is a thin-walled sac containing a watery fluid and a white spot which is the head of a future tapeworm.

The cyst of *serialis* is found under the skin of almost any part of the body, and may be as big as a golf ball or bigger. It develops slowly and is soft to the touch. Evacuation of the fluid in this cyst produces only a temporary cure. It should be removed surgically.

Tapeworms develop in the intestines of dogs which eat these cysts. For that reason all tapeworm cysts found in rabbits should be destroyed.

(E) Ear Canker

This is easily recognized, since the affected rabbits are often seen shaking their heads or scratching their ears; it is caused by mange mites. These are tiny parasites which can just be seen by the naked eye if some of the crust or scale from the ears is spread out on black paper. Under the microscope they are easily recognized. Affected rabbits can lose condition because of the constant irritation and the parasite can readily travel from one hutch to another.

There is now a satisfactory treatment for ear canker. Gammexane (B.H.C.) in any of its preparations is very efficient, and since its effects last for three weeks or more one dressing is usually all that is required. The mange mite lays eggs which are resistant to gammexane, but the young mite, which usually hatches out within 3 weeks, is very readily killed by the drug. To be absolutely certain that all the parasites are killed it is generally recommended that treatment should be repeated at the end of 3 weeks and that the bedding should be destroyed and the woodwork of the house disinfected. For disinfectant purposes a preparation containing gammexane should be given preference.

(F) Myxomatosis

This disease has not yet been responsible for any serious outbreak in domesticated rabbits in this country, but breeders should always be on the alert in case it does appear. The first symptoms is inflammation of

the eye with swelling of both upper and lower eyelids. A discharge soon appears from the eyes and then from the nose. Swelling and inflammation of the external genitals follows and in female rabbits the mammary gland may begin to secrete milk. The symptoms progress quickly and within 4-5 days of the first symptom the rabbit is moribund, the eyes and nose are gummed up by discharge, mouth breathing is prominent and small growths may appear round the nose and eyes. On post-mortem examination the internal organs appear normal apart from some congestion of the liver and spleen.

The disease is not extremely infectious or contagious, but it is fatal in almost every case. Affected rabbits should be removed and destroyed as soon as the disease is recognized. In-contact rabbits should be isolated, the bedding destroyed, utensils and cages disinfected, and since the disease can be spread by biting insects, precautions should be taken to keep out insects. At the same time vaccination of other rabbits in the rabbitry is recommended, since it does afford a certain amount of protection against the disease.

4
Hamsters

1. COMMERCIAL HAMSTER BREEDING

G. Porter

The hamster was introduced into England from the Hebrew University, Jerusalem, where a colony had been established from one female and her young discovered at Aleppo, Syria, in 1931.

This friendly little rodent very quickly became a popular pet with children. It is small, attractive, easy to handle, has little or no obnoxious smell, becomes very tame, can be kept indoors in quite small cages and presents no difficulties in feeding because the pet hamster lives quite happily on household scraps and tit-bits.

Laboratories also found that the hamster could be used for a limited number of scientific investigations. It is, therefore, bred in considerable numbers to meet these two expanding markets, yet very little investigation has been carried out on the actual husbandry of the hamster, and it would be true to say very little is known of its nutritional requirements. It is generally agreed, however, that the hamster can be treated as other small rodents whose requirements have been studied in some detail.

(A) Housing

The commercial breeder can make use of any small vermin-proof building which is wind and weather proof; such a building should have all the necessary amenities, i.e. lighting (both natural and artificial), heating, water and drainage.

(B) Caging

There are many designs of cages all of which have certain features worthy of recommendation. It should be borne in mind, however, that although the hamster is a nocturnal animal and spends most of his time asleep during the day, he is a very active little animal during the night, when he not only spends a lot of time exercising but also endeavouring to escape from his cage. The cages, therefore, must be large enough to permit him exercise and designed in a manner which will prevent his escape but still allow ample ventilation.

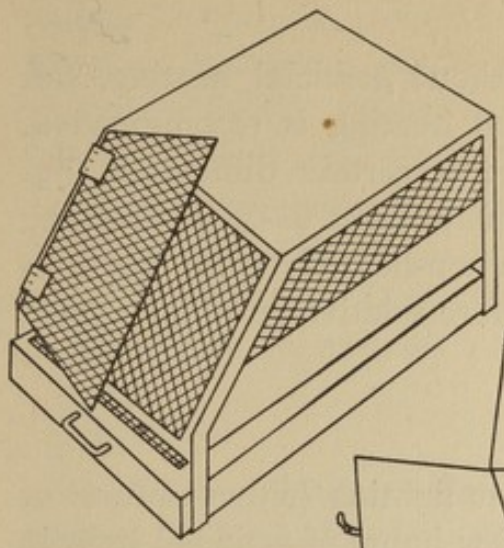


Fig. 5a.

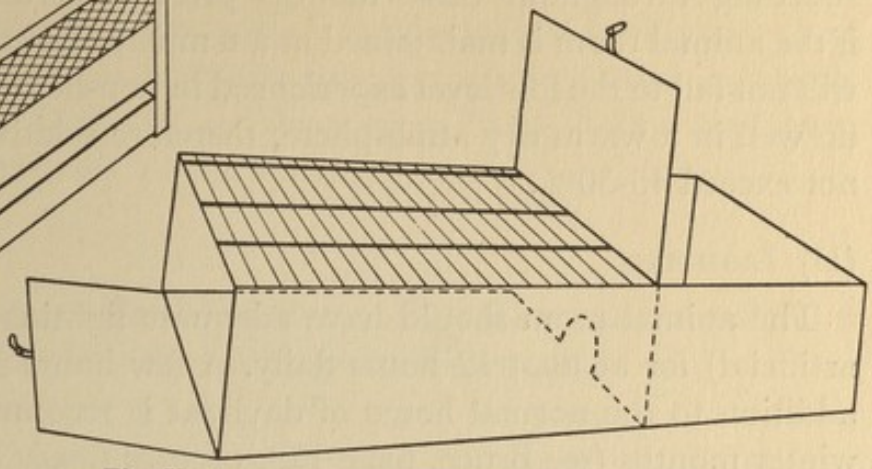


Fig. 5b.

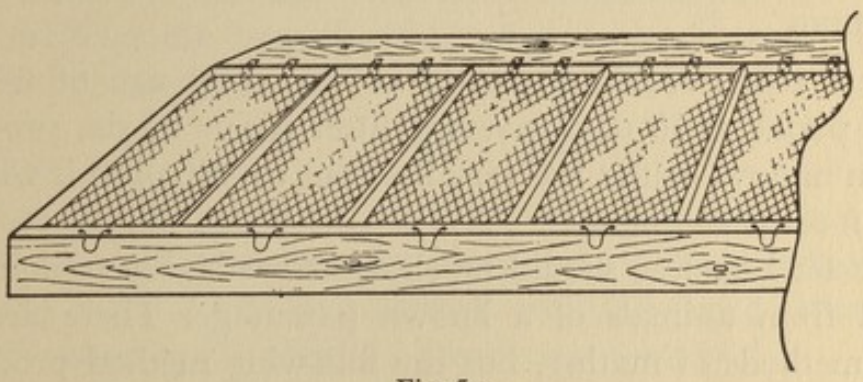


Fig. 5c.

Cages may be made out of galvanized iron, wood, resin-bonded plywood, a combination of wood or plywood and fine wire mesh.

When wood or plywood is used, the cage must be designed so that there are no corners or edges for the animals to gnaw. Lids should be made of fine mesh to allow the animal adequate light and ventilation and the lids must be securely fastened. A convenient size cage for a female and her litter should be approximately 12 in. by 6 in. by 6-7 in. high. Other dimensions may be used, but the animal should be given at least 72-80 sq. in. floor area. Stock cages for holding young animals after weaning should be approximately 12 in. by 20 in. by 6-7 in. high. These cages will hold comfortably eighteen to twenty young hamsters. Larger numbers should not be caged together, the animals do not grow so well, and if an infection should break out, control is easier when the animals are in small units. Cages where possible should be placed on racks and not built in as part of the general structure of the animal room.

(C) Temperature and Humidity

Hamsters breed quite successfully without artificial heating, but from October to March a little artificial heating is recommended. Breeding throughout these months presents certain difficulties, but if the animal room is maintained at a temperature of 68-72°F, breeding will not fall to the low level experienced in non-heated rooms. Hamsters do well in a warm dry atmosphere, therefore relative humidity should not exceed 45-50%.

(D) Lighting

The animal room should have adequate lighting (either natural or artificial) for at least 12 hours daily. A few hours of artificial light in addition to the normal hours of daylight is recommended during the winter months (see Bruce, page 129, *Common Causes of Infertility*).

(E) Breeding

Hamsters reach sexual maturity at the early age of 6-8 weeks. Gestation period is 16 days, average litter is five or six, pre-weaning losses from natural causes are very few, but cannibalism is widespread throughout some colonies.

Animals for breeding should be chosen from highly prolific healthy stock and from animals of a known parentage. There are several accepted methods of mating, but the following method produces the best results. Choose a female not younger than 8-12 weeks and a male at least 4-6 weeks older. Place the female in the male's cage during the evening and observe the animals. If the female is obviously not in oestrus and behaves aggressively towards the male, remove the female and repeat the procedure on successive evenings. When the female is in oestrus, copulation will take place; the pair may be left together for 2-3 hours, after which return the female to her own cage. Some breeders leave the pair together until the female is obviously pregnant, but this method lays extra stress on the male because the female becomes very aggressive and may savage him.

Young hamsters may be weaned when 3-4 weeks old, sexed and the males and females placed in separate boxes. Females may be re-mated when the litter has been weaned.

(F) Feeding

Hamsters drink very little water, nevertheless water should be supplied in bottles with drinking spouts. The spouts should be long

enough for the very young hamsters to reach the drinking spout, but care must be taken to ensure the animals do not build up their bedding against the drinking spout, otherwise the cage will become flooded.

Puppy biscuit meal (either dry or soaked, although the soaked meal very quickly becomes mouldy) with a supplement of fresh green food has been used successfully by many breeders. Others have used with considerable success Diet 41B and fresh green food. Puppy meal does, however, give the animals a little variety.

(G) Bedding

The floors of the cages should be given a liberal covering of softwood sawdust. Soft meadow hay should be given for nesting.

(H) Handling

This presents no difficulties, but the animals do not like to be held firmly. Always handle them over a bench or table, because the younger ones are inclined to jump.

(I) Hygiene

The cages should be cleaned out once or twice weekly, but when the young are still in the nest they should not be disturbed. When they are weaned, the cages should be scrubbed and disinfected. Water bottles should be washed and sterilized at least once weekly.

All foodstuffs should be kept in vermin-proof bins and bedding must be stored in a dry vermin-proof room.

2. HAMSTER BREEDING

C. B. Jaques

Until 1945, the golden hamster was not even heard of by the general public in this country, but traces of the animal date back long before this. In 1839 a skin and skull were discovered and G. R. Waterhouse named a variety of hamster—*Mesocricetus auratus* or Golden Hamster.

The first living specimens on record were captured in 1930 during a scientific expedition in Syria, when a female and twelve young were found in a burrow near Aleppo. These were taken to the Hebrew University at Jerusalem and, the next year, two pairs were sent to Britain. The British Hamster Club was formed in 1945 and since then a great deal of interest has grown round this little animal.

The following notes are based on my own methods and observations as a hamster breeder.

(A) Housing

To house my hamsters I use two kinds of hutches, one for mating and one for rearing. The mating box is 18 in. by 9 in. by 9 in. with a large area of wire netting at the front to give a good view of the animals. The rearing hutch is 24 in. by 12 in. by 12 in. This gives room for the hamster to rear her young in the dark or at least semi-dark. The front of this box consists of a door 7 in. wide. The remaining 5 in. act as a support for the door and for the water bottle, which is clipped to the outside.

The hutches are made of wood. This is better than metal as it keeps the temperature more constant. Many people find that the hamsters gnaw wooden hutches, but I have had no trouble in this respect. The wire in the fronts is a $\frac{3}{8}$ in. mesh. If it was larger than this, the young hamster under 2 weeks old could crawl through, and if finer the animals would not be able to climb it and would get less exercise. By having the pen 12 in. high, the hamsters get enough exercise. The hutches are kept at a temperature of 50°F thermostatically controlled.

(B) Bedding

For the bedding, any dry material can be used. I use sawdust, paper and parchment shavings from printing off-cuts.

(C) Cleaning Out

When the females are known to be mated, they are placed in a clean hutch which has been disinfected and allowed to dry out for a day. The mating pen is also cleaned out thoroughly when the doe has left it. Apart from this, it is only necessary to clean out the dirty parts of the breeding pens.

(D) Feeding

The hamster needs rather a lot of protein in his diet—about 25%. To obtain this I use best quality puppy meal as the basic food. The hamster likes variety and this is introduced by wheat, peas, beans, raisins and household scraps, including meat. This is mixed with milk and water. Oats are not fed as the husk may damage the delicate interior of the pouch. Green food or root vegetables are fed about three

times a week. Dry bread is sometimes fed as an extra and, as a special treat, earthworms and caterpillars.

I make a habit of feeding the animals at nine o'clock each evening. The food is placed in a dry or moist state at the front of the hutch and is usually cleared away by the hamster to the back of the nest. Water is given *ad lib* through drinking bottles.

Breeding does should have a good store of food in the corner of their hutch to give them a sense of security.

The hamster is very easy to feed. The food must be of good quality, but it will eat an infinite variety. In spite of this it is a choosey feeder and will reject any food that is unsuitable.

(E) Breeding

Hamsters are mated when they are 8-9 weeks old. There are two methods. The first is most satisfactory, but it may take rather a long time. The doe is only ready to accept service during a short period of time occurring once every 4 or 5 days. If this period is not at hand when she is put into the buck's hutch, the animals may fight viciously, and when this occurs the doe is returned to her own hutch. If, however, the mating is always carried out at the same time of day, there is more chance that the doe will accept service through the formation of a habit.

The second method is to put the doe into the buck's hutch for 7-10 days. A strict watch must be kept as, very often, the doe refuses to let the buck eat and they spend a lot of time fighting. The buck gets the worst of it, in fact he may soon become all skin and bone.

The gestation period for the hamster is 16 or 17 days. On an average I get litters of eight, but quite frequently I have had ten to thirteen weaned. The young are born complete with teeth and can be seen crawling to the food supply, which is near the nest, after about 10 days. They are not handled until such time as the doe fails to return them to the nest after they have been out. This is usually after about 18 days, the young are then counted and recorded.

The young can be weaned at 3 or 4 weeks. After weaning, the doe is given a week or more rest according to her condition, which depends largely on the size of the litter.

(F) Keeping Records

Records of each animal are kept on a label attached to the hutch. The records kept are as follows.

1. Identification number or letter. (Number for doe, letter for buck.)
2. Records of all matings, with time spent by doe in buck's hutch and prefix of the buck.
3. Number of young weaned and the date.

Special record is kept of does in kindle. When they have been placed in a clean rearing hutch they are given a special distinguishing ticket in addition to their ordinary record cards. When the young are born, this ticket is replaced by another card bearing the date of birth. This card normally remains with the young until they are sold, but if they are kept as replacements, they are given a number or letter and a normal record card.

3. COMMON DISEASES IN HAMSTERS[1]

The natural diseases of this relatively new laboratory species have not been studied in sufficient detail to enable a comprehensive summary to be given. It is generally accepted that the hamster is reasonably free from spontaneous disease and in comparison with other laboratory rodents is reasonably healthy. It would be wrong, however, to assume that the hamster will remain healthy unless it is provided with an adequate diet and maintained in clean comfortable quarters as recommended in the preceding chapters. Even so there will be a few current diseases, some of which may cause death while others undermine the general health of the colony. The most common diseases are described here.

(A) Intestinal Infections

1. salmonellosis

Most common of the intestinal infections, may remain undetected until reaching epidemic proportions. Diet fouled by wild rodents may precipitate the disease.

i. Visible symptoms. Ruffled coat, loss of appetite, wasting, diarrhoea, followed by death.

ii. Post mortem. Examination generally reveals congested and ulcerated intestines, enlarged spleen, abscesses on the liver, enlarged and oedematous mesenteric lymph glands, local and general peritonitis.

[1] Compiled from many sources.

iii. Method of control. Destroy all sick animals, remove and destroy all contacts, isolate other animals into small units, clean and sterilize rooms, cages and equipment. Destroy all food stocks and bedding, sterilize food bins and arrange for laboratory tests to be carried out on surviving stock.

(B) Respiratory Infections

Not common but an infectious type of pneumonia has been reported.

i. Visible symptoms. Ruffled coat, loss of appetite, rapid breathing, nasal discharge, coughing, sneezing and catarrh.

ii. Causative organisms. Pneumococcus, pneumobacillus, streptococcus and *pasteurella* groups.

iii. Post mortem. Examination generally reveals pneumonia or inflammatory changes in the lungs.

(C) Mange

A common parasitic infection, may be the direct result of bad husbandry. Highly contagious by direct contact between animals or through handling infected animals.

i. Visible symptoms. Animal in poor condition, shakes its head, scratches its ears and there is a loss of fur on various parts of the body.

Advanced stage: ears, nose and genitals covered with greyish warty scabs.

ii. Causative parasites. Notoedres notoedres.

iii. Method of control. High standard of hygiene. Avoid contact between infected and non-infected animals. Examine all animals twice weekly, always wash hands after handling each animal.

Parrish (1950) recommends treatment of the infected parts with benzyl benzoate, dimethyl-thianthrene, gammexane preparations.

(D) Leishmania Infection

Reported to be common in the hamster.

(E) Virus Diseases

Little work has been done on the natural virus infections of the hamster. A latent virus pneumonia has been reported, but the incidence is unknown.

(F) "Wet Tail"

An infection known as "Wet Tail" is fairly widespread. The animal loses condition, suffers from diarrhoea and becomes very wet and dirty around the hind quarters. Death generally occurs in a matter of days. The incidence may reach epidemic proportions. A percentage of the animals do recover; there is, however, no evidence to suggest that such animals are either immune or act as carriers. The aetiology of this disease is as yet unknown.

(G) Nutritional Deficiencies

Granados (1951), Hamilton and Hogan (1944) and several other workers have studied in detail the nutritional requirements of the hamster.

To avoid nutritional deficiencies which not only cause the classic nutritional deficiency diseases but may also precipitate others, the animals must be given an adequate diet containing all the necessary nutrients. Additional nutrients are necessary during gestation and lactation.

Commercial feeds (none of which have been designed exclusively for the hamster) should be supplemented with fresh green food, root vegetables or fruit.

The loss of weight and eventually the death of newly weaned hamsters, due to the lack of drinking-water, have been reported by the Ralston Purina Company (1961).

Obvious signs of nutritional deficiencies are: lack of alertness, loss of weight, unsteady gait, muscular weakness, poor hair coat, alopecia, polyneuritis and irritability.

Undue losses from diseases are not common in hamster colonies, nevertheless, the number of deaths which cannot be accounted for may be considerable.

Some of these deaths may be the result of injuries caused by fighting, others may be indirectly due to poor husbandry.

To maintain a hamster colony in good health, observe the following simple rules. Feed and water regularly. Clean cages at least once weekly. Maintain animal rooms at an even temperature. Wash and sterilize food containers and water bottles weekly. Scrub and sterilize cages before introducing a fresh occupant. Store food and bedding in a well ventilated vermin-proof room. Destroy all sick animals. Isolate all contacts with sick animals. Quarantine all fresh stock before introduc-

tion to established colony. Observe the highest standard of hygiene throughout the establishment.

References

Granados, H. (1951). *Acta physiol. scand.* Suppl., **87**, 1-138. Nutritional studies on growth and reproduction of the golden hamster (*Mesocricetus auratus auratus*).

Hamilton, J. W. and Hogan, A. G. (1944). *J. Nutr.* **27**, 213-224. Nutritional requirements of the Syrian hamster.

Ralston Purina Company. (1961). "Manual for Laboratory Animal Care." Supplement on hamster breeding.

5
The Rat

1. CARE AND MANAGEMENT

G. Porter

(A) Introduction

For many years the laboratory rat has made a valuable contribution to scientific research. This highly intelligent and somewhat docile rodent has been maligned and quite wrongly classed as a renegade yet it was this very renegade that Professor Hopkins used in his pioneer vitamin research at Cambridge over 50 years ago.

Since those days the number of rats used in the many fields of research has continued to increase. Lane-Petter *et al.* (1955) reported that approximately 250,000 rats were used in laboratories throughout Britain in 1952. A recent report by the Ralston Purina Company stated that about 10 million rats were used for research in the United States in 1960. It may well be, however, that with the introduction of commercially produced Specific Pathogen Free rats, the actual number of animals used will, in the very near future, be considerably reduced. This new approach to animal experimentation has been brought about by the results of recent scientific work carried out on S.P.F. and germ-free animals. In the not too distant future the pathogen-free rat will replace the conventional rat we now know, thus the number of animals used will be considerably less, but they will be rather specialized specimens. The conventional rat will, however, continue to fill a useful place in schools and, of course, as a pet.

(B) Housing (*Conventional*)

1. construction

The materials chosen should provide the maximum insulation against extremes of temperature and be impervious against vermin, i.e. cavity walls of brick, concrete or breeze blocks. The interior walls and ceiling should be finished off with a smooth surface and painted with a high-gloss washable paint.

2. floors

The floors to be constructed of a material impervious to acids and

sufficiently hard to overcome indentations caused by bins, trolleys, etc. All corners to be rounded to facilitate cleaning. The floor must slope towards a drain or gulley for easy washing down.

3. WINDOWS

The windows should, where possible, be double glazed and the size minimized to conserve heat. They must provide the maximum light yet exclude direct sunlight which is bad for the animals. Walls must be kept clear for racks, therefore windows should be placed above working level.

4. ARTIFICIAL LIGHTING AND ELECTRIC POINTS

Normal artificial lighting should be provided in a manner which will prevent a high density of light falling over the animal cages. At least two electric points should be fitted.

5. WATER

Hot and cold water supplies should be provided over one small sink unit. One cold tap with hose fitting attachment placed approximately 3 ft from the floor is convenient for washing down.

6. HEATING

The method of heating should be governed by local conditions, but to maintain an even temperature of 70°F ± 5°, the system must be thermostatically controlled.

7. VENTILATION

Air intake facilities and circulation must be considered in relation with the method of heating. A uniform distribution of fresh air is best accomplished from a single intake point which is fed mechanically through a duct and entering the animal room at ground level.

Ducts proportioned in size, evenly spaced throughout the building to provide a positive pressure within the animal rooms, will ensure the constant exhaust of fouled air. If it is impractical to install such a system, a series of thermostatically controlled electric heaters with extract fans may suffice.

8. RACKS

Wherever possible, racks should be suspended from the ceiling or fixed to the walls by brackets. They should be convertible, adjustable and easily dismantled for cleaning and sterilizing. To facilitate cleaning and hosing down, racks should not be allowed to rest on the floor.

9. CAGES

There is no standard type or design of cage, but prime consideration

must be given to the comfort of the animal. The design should be simple with adequate facilities for feeding, watering and cleaning.

Cages may be constructed of metal, plastic, wood or a combination of these materials. Metal cages demand additional animal body heat, even so when frequent sterilizing is envisaged, metal is preferable to other materials.

The wooden box is less costly, certainly less noisy and provides greater comfort for the animal than cages made from certain other

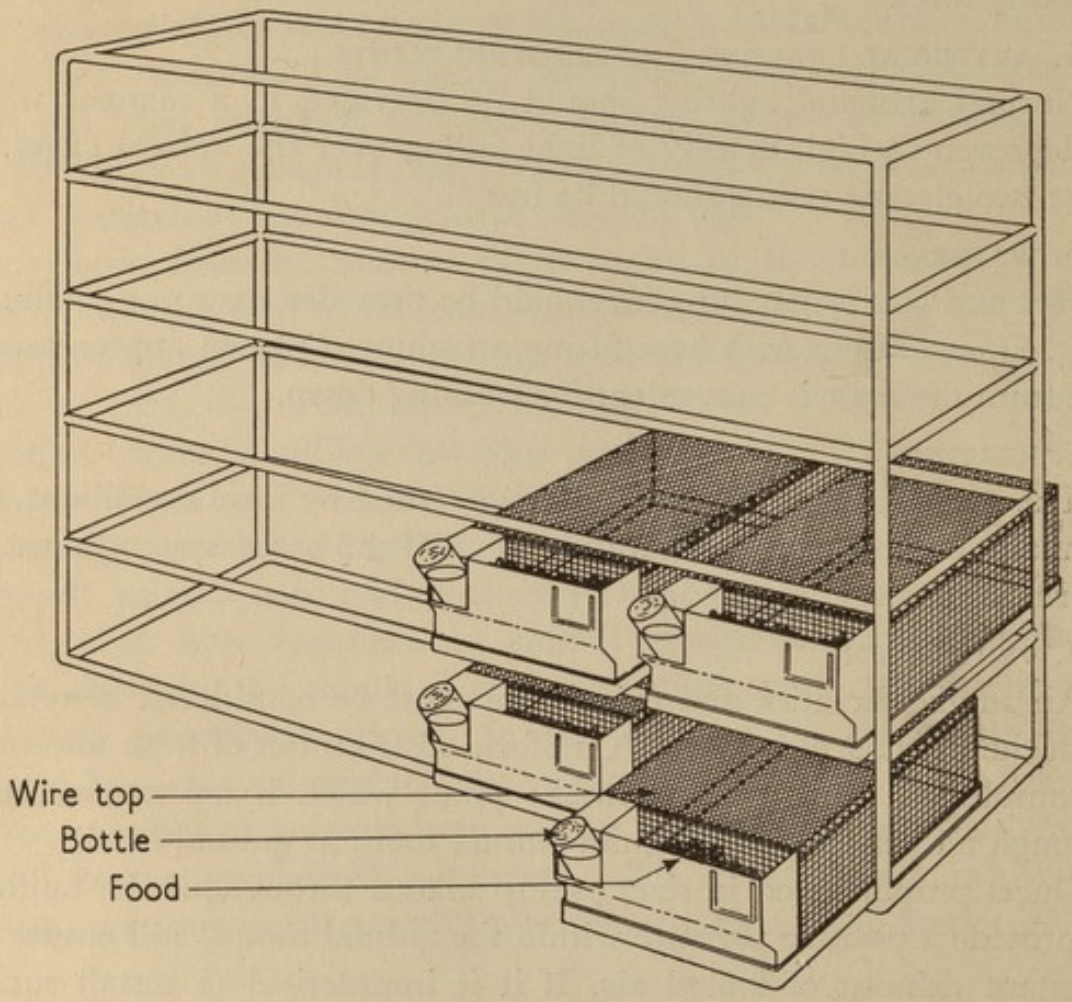


Fig. 6a.

materials, yet the wooden box is not generally accepted. There is also some controversy regarding the use of wire-mesh grids, but the question of these versus solid-floored cages depends on circumstances. Breeding stock with litters are better on solid-floored cages.

Plastic boxes which can be autoclaved are now in general use, they provide all the advantages of the wooden box, yet can be cleaned and sterilized more easily than metal.

10. CAGE SIZE

A female and her litter should be given a floor area of at least 140 in.^{2}, i.e. cage 14 in. by 10 in. by 10 in. high. A breeding pair should be given a floor area of 168 in.^{2}, i.e. cage 14 in. by 12 in. by 10 in. high.

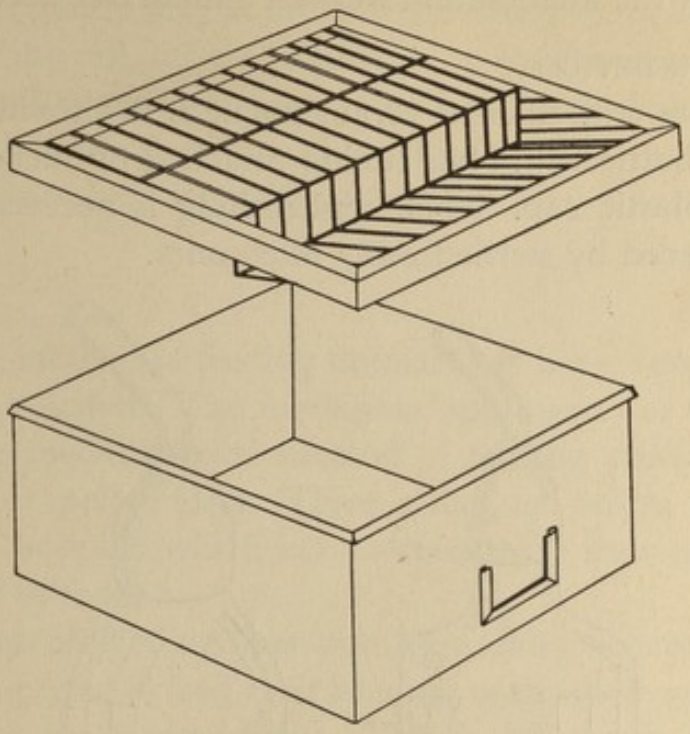


Fig. 6b.

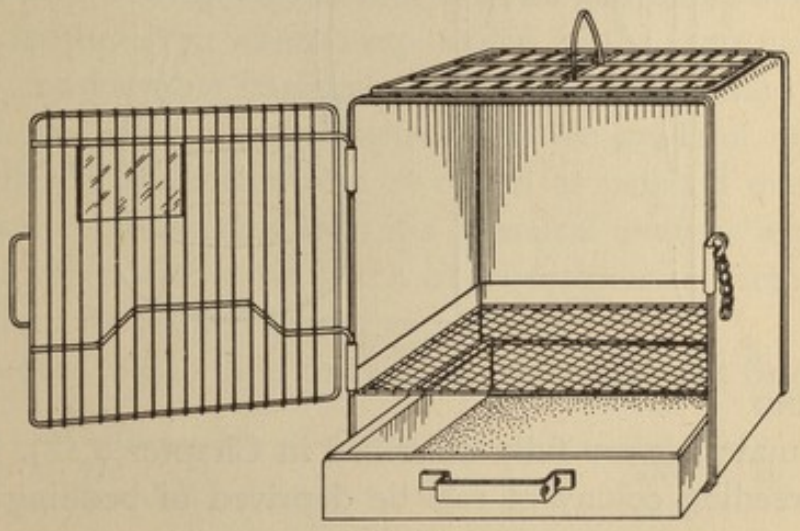


Fig. 6c.

Discretion must be used when housing groups of rats together. Young rats should be given facilities for free exercise within their cages, even if they all continue to sleep in one heap in a corner.

11. FEEDING HOPPERS

The control of infection is important in any animal colony, even

more so in a breeding colony. Food must, therefore, be given in properly designed receptacles. The receptacles or hoppers may be suspended wire baskets inside the cage or depressed wire-mesh containers incorporated into the lids of the cages. They must be designed to prevent waste, yet allow the smallest and weakest animal free access to the food.

12. WATERING DEVICES

A simple, safe and efficient arrangement is to provide water in an inverted glass bottle with glass tubing attached by means of a rubber bung or soft plastic cap. When replenishing is necessary the bottles should be replaced by sterile freshly filled ones.

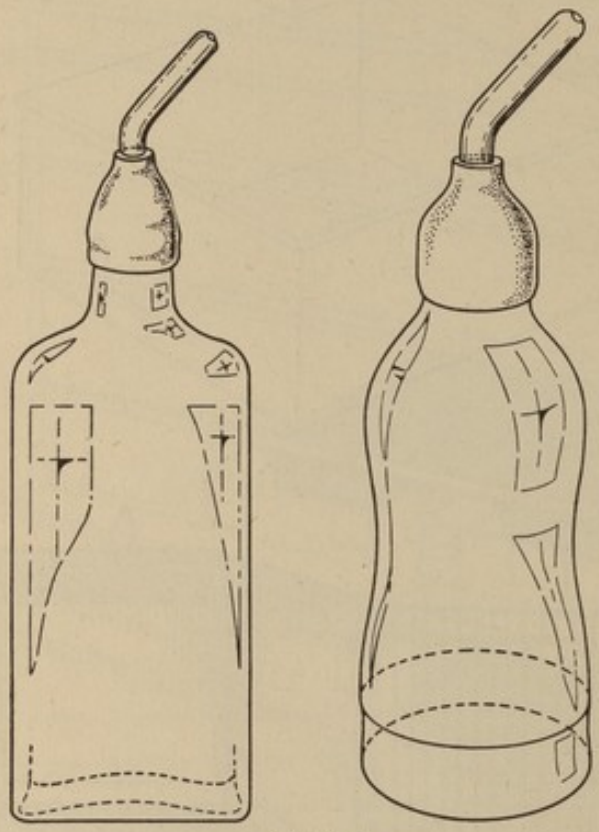


Fig. 6d.

13. BEDDING

Bedding materials are fully described in Chapter 8 (2). At no time should a breeding colony of rats be deprived of bedding or nesting materials.

(C) HANDLING

A frightened rat will always bite. The animal should therefore be given time to realize what is happening before any attempt is made at handling. It is advisable to remove the cage from the rack, place it on a bench or table, open the cage door for a few minutes, the animals will then come to the door of the cage and can be handled with ease.

A convenient method of handling is to place one hand, palm downwards, over the animal's back, slip the thumb and forefinger around the neck keeping the thumb under the animal's lower jaw, thus preventing it from biting, place the remaining fingers around the body then firmly but gently lift the animal. To handle a vicious rat, grasp it by the base of the tail, lift the animal on to the free forearm and allow it to settle for a few minutes, then handle in the prescribed manner. A lactating female must always be given time to leave her nest before any attempt is made at handling. Gloves should never be used for handling rats.

(D) Feeding

The present method of feeding animals has been developed through knowledge of nutrition. The intelligent application of this knowledge has led to the revolutionized method of feeding animals a complete diet in cube or pellet form. These cubes or pellets are offered in receptacles or hoppers which act as reservoirs, thus ensuring a continuous supply of food.

This method of feeding has not been fully accepted and many establishments prefer to feed their animals with whole grains or mashes made up from local ingredients. This is laborious, but has many advantages over a compounded diet with no guarantee that one batch is equal to another even when compounded by the same manufacturer. There is a considerable variation in the nutrition values of the raw ingredients, and even a wider variation in the grade of raw materials used by different manufacturers. A cubed or pelleted diet cannot be accepted as a complete diet even if a chemical analysis is provided by the manufacturer with each batch of food, because there is no laid-down standard for named ingredients.

The formulae of two diets in general use throughout Britain is given below.

Diet No. 41B	%	*Diet No.* 86	%
Wheatmeal	47	Wheat Whole Ground	50
Sussex Ground Oats	40	Barley Whole Ground	25
White Fish Meal	8	White Fish Meal	7
Dried Skimmed Milk	3	Meat Bone Meal	6
Dried Yeast	1	Dried Brewers Yeast	5
NaCl	1	Dried Grass Meal	5
		Cod Liver Oil	1
		NaCl	1
	100		100

These diets are normally produced in cube form, but they may also be obtained as meal and can be given as either a soft paste or mash.

Many laboratories supplement these cubed diets with milk, greens, root vegetables, meat or liver extract and various cereals. If supplements are to be given, the amounts should be worked out scientifically so that the animals are given a complete diet. Food and water are of equal importance and a captive animal should never be deprived of either.

(E) Breeding

1. foundation stock

Breeding stock must be chosen from parents with a fully recorded history. They must be healthy, vigorous, highly reproductive, possess a good mothering ability, be free of abnormalities, docile and easily handled.

2. systems of breeding

i. Pure line. A pure line is a strain of animal which has been brother × sister mated for at least twenty generations. The method of establishing and maintaining a pure line is given by Falconer, Chapter 7 (2).

ii. Closed colony. A closed colony is a group of animals maintained from within the colony and mating should be at random within the colony, from the progeny of at least fifteen breeding pairs. Rigorous selection of the animals to be randomized must be practised to prevent the breeding of animals which show effects of undesirable characteristics.

iii. Monogamous pairs. A monogamous pair is a pair of animals which remain together during their reproductive life, they also remain together during the female's gestation and lactation periods; this allows mating to take place at the post partum oestrus.

iv. Harems. A system where one male is placed with a number of females and mating takes place as and when the females are in oestrus. When the females are pregnant they are removed to single cages to produce and rear their litters, after which they are returned to the harem cage.

3. sexual maturity

The age of sexual maturity will vary considerably between strains. The environment under which the animals are maintained may also have a bearing on the age when the vagina of the female opens and ovulation takes place. Long and Evans (1922) found that both males

and females become sexually mature at about 50-60 days and as a rule the vagina opens at about 72 days. Shay (1949) studied Wistar rats and found that the vagina opened, in about 60% of the females, at 35-50 days. The testes in the males descended, in 85%, between 18-31 days. It is not, however, the general practice to mate rats until they are at least 90 days old, in fact, it has always been our practice to mate them at 110 days old. There is no sound scientific reason for this, but over the years it has been shown that breeding results were better when the females were mated at 110 days rather than at 90 days.

4. OESTROUS CYCLE

Ovulation occurs spontaneously during oestrus. The length of the cycle is about 4 days and there are five different stages in the cycle, each stage is characterized by histological changes in the epithelium of the uterus and the vagina. To define the five stages it is necessary to take vaginal smears, a technique described by Porter (1957). However, unless there is a sound scientific reason for taking vaginal smears the animals should be left undisturbed and allowed to mate at will. To check for successful matings, inspect the females early in the mornings ; if they have mated, a cornified plug will be found in the vagina, this is only evidence of a mating and not necessarily a fertile mating. Mating may take place soon after the birth of a litter because a post-partum oestrus does take place in the rat.

5. MATING PROCEDURES

The technique of mating will depend on the method of husbandry. Monogamous pairs may be placed together at a given age and allowed to mate at will, post-partum mating will also occur. In the harem system where several females are to share a mating cage it is customary to place them in a cage containing two compatible males. The use of two males is unnecessary, but it is a safeguard against the possibility of one being sterile. When pregnant, the females are placed in single cages to produce and rear their litters; the males remain together in the mating cage until fresh females are introduced.

6. GESTATION PERIOD

The length of the gestation period usually ranges from 21 to 23 days. It has been reported that the period may be extended for 7-9 days if the female is carrying a large number of young and also suckling a litter. Several workers have found that females usually produce their first litters at 21-22 days, but subsequent litters are considerably delayed.

7. PARTURITION

Providing the female is in good health there is seldom any trouble over parturition, which may last up to 1-1½ hours, depending on the size of the litter. The female cleans up each young rat as it arrives, eats the placenta which Hammett (1918) has shown furnishes a growth promoting substance to the milk.

The rat is born hairless and blind with closed ears, undeveloped limbs and a short tail. Locomotion is effected by wriggling and paddling. Development of the growing rat is rapid, the ears open at 2½-3½ days, the incisors erupt at 8-10 days, the eyes open at 14-17 days, the testes descend at approximately 40 days and the vagina opens at approximately 72 days.

8. WEIGHT AT BIRTH

The weight of young rats at birth will be governed by the physical condition of the mother, the plane of nutrition, number of young in the litter and the strain of rat. Several other environmental factors may have a bearing on size at birth, but generally a good healthy young rat will vary from 4·5 to 5·6 g. at birth.

9. WEANING

Young rats are normally weaned by age and not by weight. The young from monogamous pairs should be weaned when 20 days old, thus removing one litter before another one is born. When the female has been placed in a separate cage to produce and rear her litter, weaning may be delayed until the young are 23-28 days old. Weight at weaning will vary considerably with the number in the litter. It is the practice in some laboratories either to reduce litter size or foster young rats so that each female rears a given number per litter, thus producing a more uniform weanling.

10. SEXING

Young rats can be sexed at birth and the males can be easily distinguished by a larger genital papilla and a greater distance between the papilla and the anus. The sexes should be separated at weaning.

(F) IDENTIFICATION

Methods of permanently marking the young rat must be simple, quickly applied, easily deciphered and harmless to the animal. For short-term experimental work the animals may be marked with a

soluble stain. Consideration must be given to its chemical composition, effects, if any, on the coat and skin of the animal and whether or not it is compatible with the experiment.

For permanent marking use a chicken toe punch which can either cut holes right through the ears or a series of notches around the edges. Before attempting to mark animals by this method a well-defined code system should be drawn up.

A convenient method of marking rats is to tattoo a series of numbers on to their ears. Surgical instrument manufacturers supply tattooing outfits for this purpose. The forceps must be the smallest available to take numerals not more than $\frac{3}{16}$ in. by $\frac{1}{4}$ in.

(G) Record Keeping

There are several methods of keeping records, but elaborate time-consuming ones should be avoided. For preference a simple comprehensive system which records the necessary information should be adopted.

The success of any system of recording depends on an infallible system of marking the animals. The animal should be given a number when weaned and thereafter everything appertaining to that animal is recorded under that number. A simple card with particulars of mating and birth of litter dates, number of young born alive and other relevant information is convenient. Small cage-cards with day-to-day details should be affixed to every cage, well out of the animals' reach.

Mating record book, weaning record book, diet book are all unnecessary, unless there is every intention of using or analysing the information recorded.

(H) Humane Killing

There are several methods of killing unwanted rats, but whichever method is used, it must be carried out in a swift, humane manner.

One of the best methods of killing rats is to place them in a specially designed chamber into which coal gas or nitrogen can be introduced slowly to replace the oxygen. Such chambers should have glass panels for observation. A wire-mesh grid floor should be provided as the animals slide and then panic if placed on a polished surface.

After each rat is killed, everything must be cleared away before another one is brought on to the bench. The injection of a lethal dose of a barbiturate should be undertaken by a qualified person only.

(I) Summary

Rats should be maintained at a room temperature of 65-70°F with a relative humidity of 45-50%. The number of air changes per hour will vary under local conditions, but should be 6-8 per hour.

A high density of artificial light is bad, as is strong sunlight. The animals should, however, have at least 12 hours light daily throughout the year. Food and water must be available at all times. Bedding must always be provided in the form of soft wood sawdust or peat moss litter, nesting material must be provided for pregnant females. The normal body temperature of the rat is 37·5°C or 99·5°F with a respiration rate of 210 per minute. The oestrus cycle is 4-5 days and the mating age is normally from 90-110 days with a gestation period of 21-23 days. Young rats may be weaned at 21-28 days.

References

Hammett, F. S. (1918). *J. biol. Chem.* **36**, 569.

Lane-Petter, W., Barber, A. Pamela and King, Hilary J. (1955). *Brit. vet. J.* **3**, 7, 282-299.

Long, J. A. and Evans, H. M. (1922). "Memoirs", Univ. Calif. **6**.

Porter, G. (1957). "UFAW Handbook on the Care and Management of Laboratory Animals" (Ed. Worden, A. N. and Lane-Petter, W.), Chap. 32.

Ralston Purina Company (1961). "Manual for Laboratory Animal Care."

Shay (1949). Personal communication reported by E. J. Farris. "The Rat in Laboratory Investigation" (Ed. Farris, E. J. and Griffiths, J. Q.), Chap. 1.

APPENDIX

Specific Pathogen Free Rats

G. Porter

The term specific pathogen free, practically unheard of ten years ago, is today the accepted definition for an animal which is known to be free from commonly occurring pathogens.

Reyniers (1946) and Gustafsson (1948) demonstrated methods of producing and rearing germ-free rats, and these methods, with some modifications, have now been used for the establishment of specific pathogen free colonies. Confusion still arises over the terms germ-free and specific pathogen free. There is some similarity between the two, but only so far as both terms apply to rats which have been derived from caesarean section. The germ-free animals after caesarean section remain in a piece of specially designed apparatus known as a germ-free isolator, that maintains them in a sterile condition throughout their lives. These conditions do impose limitations on the uses to which the

germ-free rat can be put. The specific pathogen free rat on the other hand is maintained in a conventional environment and laboratories may, therefore, establish such colonies as a matter of routine.

In the United States of America enterprising commercial animal breeders, like Wentworth Cumming (1961) and Foster (1961), have shown that it is possible to produce on a large scale S.P.F. mice and rats. They have also found that, excluding the initial expense, it is more economical to maintain and use S.P.F. animals than to maintain and use what are now known as conventional animals.

1. BUILDINGS

The cost need not be prohibitive if careful consideration is given to the design and the materials used. One must, however, bear in mind that the building is for a special purpose and allowances have to be made for the provision in this building of complete specific pathogen free barriers, complete acclimatization, observation vestibules and other features which Foster (1961) describes as identical in principle to the germ-free isolator.

2. PERSONNEL ENTRANCE

This should be made through a number of hallways or vestibules, the entry to each one being controlled by closing the previous door, thus it should be impossible to proceed beyond the immediate entrance before the outer door is closed. These hallways are designed as air locks, fitted with U.V. lighting, insecticidal and bactericidal aerosols, and should provide facilities for removing outer clothing, footwear, etc. The final hallways are for the removal of all clothing, entry is then made through a shower bath. Sterile clothes are provided after showering. Exit is the reverse action. Other methods have been installed but in general they are similar.

3. GOODS ENTRANCE

Equipment, clothing and all other items must pass through either an autoclave or a specially designed gas chamber, thus ensuring complete sterilization before entering the building.

4. FOOD

S.P.F. food does present a problem as autoclaving may destroy certain nutrients and ethyleneoxide for various reasons cannot be used to sterilize foodstuffs.

Several methods of supplying S.P.F. diet have been described. Cumming (1961) has food delivered inside several wrappings, the outside wrappings are removed and the diet is then passed through a

hatchway into the clean side. This may not be ideal, but at least Cumming has attempted to overcome the problem of feeding a large S.P.F. colony without the added expenditure of having special diet delivered in hermetically sealed tins. Further development of a method used by Sabourdy (1961), who feeds a baked diet to his conventional colonies, may be worth considering. If a special oven was arranged with a door on each side of the barrier, then providing sterilized diet would present no problem. Velaz, in Czechoslovakia, have successfully bred mice on a baked diet containing the following ingredients:

Feed	%
Wheat (whole ground)	66·19
Dried full cream milk	9·53
Casein	14·31
Dried lucerne meal	1·447
Calcium carbonate	1·47
Cod liver oil	0·74
Butter (margarine)	4·715
Salt	0·234

Irradiation of the diets could be considered as a possibility as also could a method of pasteurizing the meal before passing it into a cubing machine on the clean side of the barrier.

A lot of research is still necessary on methods of supplying an S.P.F. colony with an adequate sterile diet. Water considered fit for human consumption should be adequate for S.P.F. animals, if however, there is any doubt distilled water may be given.

5. EXIT FOR ANIMALS

Animals should be placed in sterile boxes with sterile bedding and passed out through an airlock system of chutes.

6. DISPOSAL OF WASTE MATERIALS

For excreta and other waste material chutes have to be specially designed to ensure that they open only one way and that traps are incorporated to ensure no entry from beyond the barrier. Waste material may be fed through a non-return chute direct into the incinerator.

7. CLEANING CAGES AND EQUIPMENT

This should be carried out in a room set aside for the purpose, the method employed to be as recommended for a conventional colony.

8. FOUNDATION STOCK

Foundation stock may be obtained from an existing S.P.F. colony or

by caesarean section from the existing stock. The pups must be either hand reared inside the S.P.F. barriers or fostered on to S.P.F. females supplied from a reliable S.P.F. source; the latter method is preferable as hand rearing pups entails a 24-hour attendance for at least 14 days. The S.P.F. foster mothers must be transferred in specially designed S.P.F. containers.

9. BREEDING

The techniques recommended for the conventional colony apply equally to the S.P.F. colony.

REFERENCES

Foster, H. L. (1961). *J. Anim. Tech. Ass* **12**, 64.
Wentworth Cumming, C. N. (1961). *J. Anim. Tech. Ass.* **12**, 75.

2. COMMON DISEASES OF THE RAT[1]

The domesticated rat is far from the healthy animal we have been led to believe.

Intestinal and respiratory infections occur with alarming frequency in most conventional colonies, yet the actual number of deaths which are investigated and finally attributed to these infections is few.

(A) INTESTINAL INFECTIONS

Paratyphoid disease caused by the Salmonella group of organisms is not uncommon in rat colonies, as in other laboratory animals.

i. Causative Organisms. Salm. enteritidis and *Salm. typhimurium.*

ii. Visible symptoms (*Acute type*). Loss of weight, poor, ruffled coat, scabs about the nose, pale, anaemic-looking animals, diarrhoea, usually severe anaemia followed by death.

The *chronic type* is much more common but usually less severe. The infected animals may look ill, which suggests a carrier condition when the disease is passed on to other animals.

iii. Post mortem. Examination usually reveals pneumonia and haemorrage of the lungs, enlarged and congested spleen. Intestines may be inflamed with blood-stained contents. The lymphatic tissue in gut may show signs of ulceration. The liver generally pale, friable and possibly necrotic.

[1] Compiled from many sources.

iv. Method of control. Maintain a high standard of hygiene, ensure foodstuffs have not been contaminated by wild rodents. Destroy all sick animals, search through stock for possible carriers.

(B) Respiratory Infections

The respiratory infections include pneumonia, bronchopneumonia and middle ear disease.

1. pneumonia

Common in most colonies, high incidence in adult rats, may be induced by sudden drop in temperature. Generally acute, death occurs in a matter of days.

i. Causative organisms. Haemolytic streptococci, *Haemophilus bronchisepticus*, *Streptobacillus moniliformis.*

ii. Visible symptoms and post mortem examination. Similar to pneumonia in mice. See Chapter 2 (4).

2. bronchopneumonia

A chronic infection, common in most colonies. Animals generally in poor condition with frequent sneezing and coughing.

i. Causative organisms. Probably a virus and/or pleuropneumonia-like organism.

ii. Post mortem. Examination generally reveals grey translucent spot or nodules in the lungs, otherwise little abnormalities may be detected.

3. middle ear disease

Fairly widespread throughout most colonies, may have some connection with respiratory infections. Generally affects older rats but may also be found in young ones.

i. Visible symptoms. In the early stages slight tilting of the head. In advanced stage animal runs in a circle, staggers, falls and has difficulty in regaining its balance. When suspended by the tail the animal rotates rapidly. (This practice is both unnecessary and cruel.)

ii. Causative organisms. Streptobacillus moniliformis and pleuropneumonia-like organisms are possible. Exactly how these organisms come to effect the middle ear has not been determined.

iii. Post mortem. Examination generally reveals pus in the middle ear, inflammation extending to the labyrinth, inflammation of the upper respiratory tract. A small percentage of cases show infection in the tympanic cavity.

iv. Method of control. Remove and destroy all infected animals.

(C) MANGE OR RAT SCABIES

Almost entirely due to bad husbandry, especially where the animals are kept in unhygienic conditions. Readily transmitted to other species and also to humans.

i. Visible symptoms. Grey watery lesions around the ears, nose and the root of the tail.

ii. Method of control. Maintain a high standard of hygiene, regularly clean and sterilize cages and equipment. Examine animals frequently.

Local treatment. Apply benzyl benzoate, dimethyl-thianthrene or gammexane preparations.

(D) ENDOPARASITES

Few parasitic worms infect laboratory rat colonies. Contamination of food and bedding either in transit or in store can, however, lead to a fairly wide distribution of cysts of the cat tape worm (*Taenia crassicollis*).

i. Visible symptoms. Animals in poor condition. There may be considerable abdominal swelling.

ii. Post mortem. Maintain a high standard of hygiene, sterilize all bedding materials and food if facilities are available.

(E) NUTRITIONAL DEFICIENCIES

Gross deficiencies are always obvious but an imbalance or a borderline deficiency may take several generations to become apparent. Bruce, Chapter 4 (2), draws attention to the fact that dietary requirements of essential factors are a question of balance rather than of fixed amounts, this being so the majority of stock diets provide the essential nutrients in the correct proportions. Gross nutritional deficiencies are therefore exceptionally rare, but the evidence of vitamin deficiencies is apparent in many colonies. The effects of such deficiencies on fertility are very fully dealt with by Bruce.

6

Feeding

1. DIETS FOR LABORATORY ANIMALS[1]

F. J. Dyer

It is common knowledge that every creature must eat to live. Dietetics, the study of food and feeding, is now an accepted branch of applied science. The students of dietetics are a very mixed company, and among them the animal breeder claims an honourable place.

The stoker feeds the furnace, which is expected to burn. Its work is to give out energy as heat. The animal stokes up its body in order to be able to work, and the work it is able to do is evidence of the stored energy being burned up or expended.

Now, in order to express itself exactly every applied science needs its units of measurement: feet and inches for the carpenter, watts and kilowatt-hours for the electrician, degrees of temperature for the stoker. Similarly the science of dietetics has its scale of measurement by which to assess the value of the food eaten by the animal. Calories are the units for measuring food values.

Just as a miner needs more calories per day than does a civil servant, so an energetic animal like the dog requires more calories, body weight for body weight, than the guinea-pig. Therefore its diet must contain proportionately more calories.

A Calorie, spelt with a capital C, is the amount of heat required to raise 1 kilogram of water through 1 degree centigrade. Physicists have measured the Calorie values of many of our commonly used foodstuffs and compiled reference tables of these values for our guidance. Before looking at these tables, however, we must consider a few fundamental concepts about food in general, and animal feeding-stuffs in particular.

(A) The House of Food and its Bricks

The essential difference between a plant and an animal is that a plant can make its own complicated food-components out of simple ingredients found in the soil and the air. Broadly speaking, the animal cannot build up, or synthesize, it can only break down complex substances into simpler ones, which it digests and uses. Food is used to

[1] Revised by G. G. Grindley.

supply energy for movement, respiration, digestion, growth and reproduction, and to make good the tissues exhausted in life's battles. In speaking of the house of food, we are therefore thinking of a very busy factory, with its stores, machinery and repair sheds.

But first let us look at the bricks from which our house is built. They must above all possess the right shape, consistency and colour. There are different classes of bricks, called by scientists Carbohydrates, Proteins, Fats, Minerals and Accessory Factors, or Vitamins. Let us now briefly examine these.

1. WHAT ARE CARBOHYDRATES?

A short answer to this question would tell us that they are starch and sugarlike foods, and, in the case of some animals, cellulose. To give a more satisfactory answer, however, it will be necessary to introduce some simple chemical language.

Matter is composed of Elements. That is to say, when we break down complex matter, like a piece of sugar, into its ultimate components, so that by ordinary methods of chemical analysis these will not yield anything simpler, we have arrived at elements. There are about one hundred of these elements; some are very familiar ones like oxygen and nitrogen, which we breathe. Others like hydrogen and helium are lighter than air and keep balloons afloat. Still others, like carbon (charcoal and diamonds are both composed of this element), are solids. If nature expends energy upon several elements, they blend harmoniously and in definite regular proportions, to form Compounds. By chemically blending together black solid carbon with the invisible gases hydrogen and oxygen we get the white solids sugar and starch. The chemist expresses the last sentence in shorthand symbols each having a quantitative meaning as well as being a label for an element.

$$6C + \underbrace{6H_2 + 3O_2}_{\text{GASES}} \xrightarrow{(+\text{Energy})} \underset{\text{SUGAR}}{C_6H_{12}O_6}$$

CARBON	GASES	SUGAR
black, insoluble	invisible, very slightly soluble	solid, sweet, white, *very* soluble

It must not be thought that this change is brought about readily. In nature the energy required to effect the synthesis of sugar from carbon, hydrogen and oxygen comes from the sun, assisted by numerous other agencies.

A substance resembling, in analytical pattern, the one shown is termed a Carbohydrate. It will be noted that the elements hydrogen

and oxygen are present in the proportion of two to one, or in simplest symbolic terms H_2O, which is, as everyone knows, the chemical formula for water. So a carbohydrate is a member of a group of complex chemical substances, resembling starches and sugars, which is composed of the elements carbon, hydrogen and oxygen, and has hydrogen and oxygen present in the same ratio (2:1) as they are in water (H_2O).

Some well-known food carbohydrates are glucose and fructose, present in sweet fruits, such as grapes and figs; sucrose, which is found in the seeds and leaves of plants and is the most abundant of the sugars (we obtain most sucrose from sugar cane and sugar beet); starch, which comes from cereals, and glycogen, or animal starch.

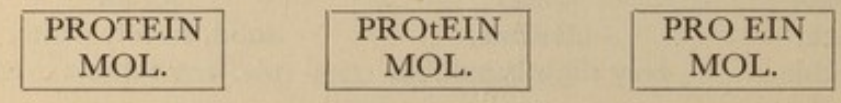
The published analysis of Pellet Diet 18 (Bruce) shows that it contains 33·7% carbohydrate.[1]

2. PROTEINS

A brief definition of this type of food-brick is "muscle-building, nitrogenous food". Common food proteins are lean meat, fish, egg white, gelatin, blood meal and pulses (peas, beans, lentils, etc.). A closer examination of the protein bricks reveals their complicated patterns. Proteins themselves are built up from a number of simple bricklets, called amino acids, dovetailed into one another.

A first-class protein contains all the essential bricklets (amino acids) in approximately those proportions required by the animal. A second-class protein may contain all the essential amino acids, but in the wrong proportions, so that the animal, unable to make its own amino acids, has to eat larger quantities of such a protein in order to obtain the necessary minimum of those amino acids poorly represented in it. In some proteins there is a total deficiency of some essential amino acids, and no amount of overeating will make good this deficiency.

Professor Catchart—a famous dietitian—once illustrated this point by three diagrams.



I. Good II. Inadequate III. Deficient

Figure I represents a first-class protein; in figure II, the shortage of bricklet "T" could be repaired by giving more of the protein; in

[1] Bruce, H. M. (1947). *J. Hyg., Camb.* **45**, 169. Feeding and Breeding of Laboratory Animals (IV): Breeding Rabbits without fresh Green Food.

figure III excess feeding of the whole brick will never supply the missing bricklet.

Proteins, unlike carbohydrates, contain the element nitrogen, as well as carbon, hydrogen and oxygen. When protein is fed to an animal, for example as casein in dried milk, the digestive processes of the body must first, with the aid of enzymes, split up the large insoluble brick into the small bricklets (amino acids). These are soluble and can therefore be readily absorbed into the blood, to be used either for the building up or the repair of tissues.

In Pellet Diet 18, to which reference has already been made, there is present 16·5% of digestible protein.

3. THE FAT BRICKS

Fats which are used as foods are drawn from sources in both the vegetable and animal kingdoms. Those which are liquid at ordinary temperatures are called oils, for example olive and cod liver oil, the term fat being reserved for those which are solid in average temperate climates, for example, butter, suet and lard. It is interesting to observe how widely nature distributes reserves of oil and fat; both in seeds, fruits and roots; and as varying amounts of *adipose* tissue in animal and human beings. Evidently fat in one form or another plays an important part in living organisms. It has a protective function, like the blubber which enables a whale to keep warm in arctic seas. It also acts as a convenient storehouse for energy.

The simpler forms of oil and fat, like the carbohydrates, consist of the elements carbon, hydrogen and oxygen, though in different proportions. As everyone knows, oil and water do not mix, which is another way of saying that oils and fats are insoluble in water. Despite the fondness of many people for fats (witness the great popularity of fish and chips), they are less readily digestible than other forms of food. Nevertheless, fat is a very necessary ingredient of a complete diet and the digestive processes of the intestine, aided by the bile, render fat soluble and so enable it to be absorbed by the blood and utilized by the body. The process resembles washing up greasy plates where water alone is useless; water plus washing soda is effective.

In the animal's intestine, alkali of the digestive juices, together with bile, brings about this process in two stages; firstly, the fat becomes emulsified, that is to say, its large particles are finely divided into very small ones, resembling a cream; secondly, the alkali, helped by an enzyme, splits the fat into a soluble "soap" and glycerine.

The process can be stated in this way.

$$\text{FAT (insoluble)} \xrightarrow[\text{from the digestive juices}]{+\text{water}+\text{alkali}} \text{SOAP (soluble)} + \text{glycerine}$$

The "soap" can be readily absorbed through the wall of the small intestine into the blood. It is then carried to depots, where it remains until it is needed by the body.

In addition to these simple fats, animals require fatty substances for the maintenance of the nerve and brain tissues. One of these, Cholesterol, is closely allied to vitamin D and is present in all body cells. Another is Lecithin, containing both phosphorus and nitrogen, present (10%) in egg yolk and (2%) in liver and blood. Another group of fat-like waxy compounds may be regarded as bricks which have been fashioned and sculptured to fulfil very special functions. In Pellet Diet 18 there is present 4·6% fat.

Two other classes of dietary bricks remain to be described, Minerals and Accessory Factors. So far we have considered carbohydrates, protein or nitrogenous ingredients, and fats. It will be remembered that earlier we contrasted elements with compounds, which are the result of the intimate chemical union of two or more elements. Compounds will figure prominently in our study of minerals.

4. MINERALS

Perhaps to the lay mind, the word mineral conjures up an image of a mine from which base or precious metals are won from ore. Mineral waters are often described as "soft drinks". This name has an indirect relevance to our present problem. Dietary minerals are usually metallic compounds, either mined directly from the earth and then highly purified, like rock salt, or made in the chemical manufacturer's laboratory, like potassium hydrogen phosphate. The mineral component of soft drinks (and some others), is the non-metallic gaseous compound, carbon dioxide (CO_2), together with traces of appetizing mineral salts.

What does the chemist mean when he uses the word "salts"? We know what is meant by table salt, namely a specially dry form of sodium chloride (NaCl), while "salts" frequently connotes an aperient such as Epsom Salts (magnesium sulphate), Glauber's salts (sodium sulphate), or a proprietary brand of liver salts. Their common characteristic is that they contain one or more mineral compounds.

A salt, then, used in its chemical sense means that compound

formed by the intimate chemical union of a metallic element with one or more non-metallic elements. Without salt, food tastes flat and unappetizing; without mineral salts blood would not work its miracles of revitalizing tissues, of removing waste products or of making the brain and nerves respond to stimuli.

In healthy (land) mammals the amount of sodium chloride (NaCl) consistently present in blood plasma lies between 0·8 and 0·9%, and any deviation from the normal average for the species indicates ill-health in one form or another.

As blood moves round the body pervading almost every cell, these mineral salts undergo a certain (limited) amount of interchange with those of the tissues visited. Hence in time "blood minerals" would become spent unless renewed through the animal's food.

A few of the other essential mineral compounds required by an animal, and therefore included in dietaries, are given below; it will be seen that a metallic element (M) is in each compound united with non-metallic elements (NM).

M	*NM*
POTASSIUM	CHLORIDE
IRON	SULPHATE
MAGNESIUM	CHLORIDE
CALCIUM	CARBONATE
POTASSIUM	HYDROGEN PHOSPHATE

We have already mentioned the following elements: hydrogen, oxygen, nitrogen, sulphur, carbon, iron, magnesium, chloride, phosphorus, sodium, potassium, calcium and magnesium. Trace elements, which are popularly less well known, include iodine, fluorine (for healthy teeth), manganese, copper, cobalt, and others all of which are essential to the health of the living body.

Bones and teeth will flash into the mind as typical mineral-like structures: hard, durable, insoluble in water. Everyone knows that bones are made from lime (calcium), but fewer people realize that the mineral content of bones and teeth need to be constantly renewed by the blood stream, which in turn gets its supplies (partly at least) from the food.

The interplay of mineral components and accessory factors will be studied later; suffice it to say that if there is any disharmony among these classes of brick, instead of the blood passing on minerals to the bones, it actually robs them of some of their store, causing them to

become brittle and porous. This happens in rickets and in certain deficiency diseases, which attack the aged.

Apart from their functions of fabric-building and of circulating supplies, dietary minerals also help to provide chemical buffers. As this name suggests, these act as a cushion between more violent elements, some too acid, others too alkaline, which would entirely upset the delicately balanced blood reaction.

Years ago Claude Bernard, the French physiologist, spoke of "the fixity of the internal environment" as the basis of physiological health. We have seen that blood (part of the internal environment) is well buffered towards producing this fixity by reason of the minerals ultimately supplied to the animal in its food.

Diet 18 in addition to any minerals present in its other components includes 1% each of calcium carbonate and sodium chloride, added deliberately.

5. ACCESSORY SUBSTANCES (VITAMINS)

Although the bricks about to be described are very minute components of diet, nevertheless they are essential ones and their lack in the foodstuffs of animals causes diseases. These accessory food factors are the well-known group called vitamins, distinguished from one another by capital letters thus: A, B_1, B_2, B_6 (and latterly) B_{12}, C, D_2, D_3, E and K. These are known to be essential for the complete well-being of the larger species of laboratory animal. Some animals like the rat can do without vitamin C, but for the guinea-pig this factor must be supplied in the diet; otherwise, scurvy will result.

A brief review of the earlier history of, and recent developments in, our knowledge of vitamins may induce the reader to read more about the fascinating story of these important dietary bricks.

In 1912 Professor Gowland Hopkins at Cambridge found that although he gave his rats and mice a diet complete as regards all the then-known essential components, their growth curve flattened out after a few weeks. Kindred animals similarly reared, but with a daily addition of approximately one teaspoonful of fresh milk per animal, grew and continued to grow long after their milkless brethren had steadied in weight. The milk was supplying minute traces of essential accessory factors. The particular factor which concerned Hopkins' colony is now known as vitamin A; in those pioneer days it was obtainable only in the form of concentrate associated with one part of the fat (the non-saponifiable fraction, as chemists call it). Today, vitamin A can be

synthesized by chemical means. Vitamin A dissolves in fats and oils, but not in water, and in this respect resembles the vitamins D (both D_2 and D_3) and vitamin E. They are therefore termed fat-soluble vitamins; by contrast, the B-vitamins and vitamin C are water-soluble vitamins.

Vitamin A promotes normal growth, healthy mucous membranes, adequate visual-purple in the eye, thereby preventing night blindness, and (with vitamin E) guarantees the regularity of the onset of heat, or the oestrus cycle, in rodents. A summary of the nature, properties and common sources of the vitamins, chiefly applicable to laboratory animals will be found in Table I.

(B) The Mortar for the Bricks

We have now five types of brick, and if we put these together in the correct proportions we should get a symmetrical structure, or, technically, a balanced diet.

The diets described by Parkes and others at Mill Hill now available in cubed form are examples of well-balanced diets. In fact, the M.R.C. workers claim that for certain species of animal living under ideal animal-house conditions, these diets need no supplements—except water.

It may therefore be said that the fluid compound water constitutes the mortar for the bricks, in order to build the ideal dietary edifice.

Not only does water quench the thirst, but in so doing it supplies to the body the medium in which minerals, salts, some carbohydrates, water-soluble vitamins, and gases dissolve, thereby making solutions. Only in solution can chemical reactions take place. Plant and animal life, with all its manifest evidences of growth, movement, digestion, excretion and reproduction, consists of well-arranged chemical reactions taking place in a watery solution.

(C) Calorie Values of the Bricks

Every unit of carbohydrate, fat and protein contributes its quota of calories[1] to a diet. By direct physical measurements these calorie values have been measured and recorded for the use of dietitians. Agreed average values are as follows. One gram of carbohydrate yields 4·1 calories, one gram of protein yields 4·1 calories, and one gram of fat yields 9·3 calories.

A word of warning should be spoken against regarding calorie value as synonymous with the biological value of any particular component.

[1] 1 Calorie equals 1000 calories.

TABLE I. The Vitamins

Vitamin	*Other names*	*Sources* *Rich++* *Moderate+*	*Stability (keeping properties)*	*Effects of deficiency*	*Daily needs (human)*
A	Anti-Infective Vitamin (Axerophthol)	Animal fats+ Cod and Halibut liver oils++ As Carotene in vegetables Spinach++	Fair; readily oxidized Keep in cool dark place	Growth retarded Eyeballs keratinise Night blindness Oestrus cycle interrupted	2 mg= 6,000 1 nt Units
B_1	Aneurin Thiamin } Chloride	Cereals (husks)++ Yeast, liver+ Egg yolk+	Stable but destroyed by boiling and alkaline	Beri-beri Neuritis, constipation Loss of appetite	1·5 mg 500 I.U.
B_2	Riboflavin	Yeast++ Bran, milk++ Liver+, kidney+	Affected by sunlight and alkalies, otherwise stable	Upsets carbohydrate metabolism Retards growth	3 mg
	Adermin, Pyridoxine	Synthetic	Stable	Swollen paws, ulcerated tongue; epileptiform fits.	150 mg
—	Nicotinic Acid (P—P factor)	Yeast+ Liver+ Milk+ Meat+ Egg+	Very stable	Pellagra (Eastern disease) Dermatitis	50-100 mg
—	Pantothenic Acid	Most animal tissues, especially heart and liver	Calcium salt, stable	Upsets enzyme activities; hair greying in rat; spinal cord defects.	100 mg
B_{12}	Anti-Anaemia Vitamin	Liver++ Bacterial extracts	Very stable	Causes pernicious anaemia	·001 mg

TABLE I.—*continued*

C	Ascorbic Acid	Fresh citrus fruits (lemons, oranges) Tomatoes, turnips+ Green vegetables+ Blackcurrant+ Rosehips++ walnuts+	Unstable; must be kept in *acid* medium	Impairs red blood cell formation: capillary haemorrhages; scurvy. Many tissue oxidations and healing of wounds delayed.	60 mg (1,200 I.U.
D_2	Calciferol	Irradiates Ergosterol (from a fungus)	Stable Withstands cooking	Rickets, dental caries Impairs uptake of calcium and phosphorus, in mammals	0·012 mg (500 I.U.)
D_3	—	Irradiated milk fat Animal and fish liver oils++	Heat and oxygen resisting.	In the chick—leg weakness and rickets.	0·012 mg (500 I.U.)
E	Tocopherol	Wheat germ++ Egg yolk+ Lettuce+ Milk+	Withstands heat, but readily oxidized.	Degeneration of epithelium in reproductive organs: sterility; abortion.	5 mg (5 I.U.)
K	Naptho-quinones	Alfalfa grass+ Pig liver fat+ Soya: green vegetables	Stable	Haemorrhages (because the blood-clotting is inhibited).	5 mg

Notes to Table

1. Vitamins D_2 and D_3 are both present in cod liver oil (especially the less refined varieties used for poultry). Only D_3 prevents chick leg weakness. Rats and mice can utilize both forms of the vitamin.
2. Vitamin B_{12} has been recently prepared synthetically. There is nothing parallel to pernicious anaemia known in animals, so the use of this (precious) vitamin is, so far, confined to human subjects.
3. Many of the discoveries connected with vitamin deficiencies have been made on the smaller laboratory animals: rat, guinea-pig and chick. The knowledge is now widely and successfully applied to domestic pets, cattle and pigs, and in human therapeutics.

As Professor Cathcart points out, cellulose, a regular component of all vegetable feeding-stuffs, and gelatine, a protein obtainable from bones, each gives the standard calorie value for its class; neither is a perfect brick in respect of biological food-value.

With this proviso, however, calories are useful indicators of the requirements of a body. For a fully grown man weighing 11 stones (= 70 kilograms) it is agreed that (*a*) asleep, he needs 62 calories per hour, (*b*) awake but at rest he burns 68 calories per hour, (*c*) doing active work he requires 168 calories per hour.

For 8 hours each of sleep, work and restful leisure the total calorie requirement for a man is therefore between 2,700 and 3,000 per day. The figure of 3,000 was accepted by a Scientific Food Commission which met in 1915, but today a slightly higher figure is preferred to allow a margin of safety. Calorie requirements for laboratory animals are worked out partly on their body weights, and partly according to their mode of behaviour, whether it be sluggish or sprightly.

Some experts calculate calorie requirements from the surface area of an animal, others upon a relationship between weight and height. Assuming that man, rabbit, guinea-pig and mouse were all of the similar temperament and habit, their daily calorie requirements would be approximately as follows.

Type	*Weight* (g)	*Relative surface area* $(cm)^2$	*Calorie requirement (per day)*
Mouse . .	27	9	20
Guinea-pig .	350	50	100
Rabbit . .	2,200	170	350
Man . .	64,000	1,600	3,000

In fact, a mouse consumes its own weight of food in 4-14 days; a man does not, and need not!

(D) The Meaning of a Complete Diet

The house of diet has now been surveyed and the different types of brick named and described. The mortar for the bricks consists of water and roughage; water, because the body is a vast chemical laboratory and all chemical reactions take place in a solution. Roughage is needful, because although we can imagine a diet complete as regards bricks and mortar, there our analogy with a house ceases. The animal is not to live *in* this house (or keep it vacant), but to live *because* of it.

Digestion of food presupposes assimilation, and this process depends on moving the food through the digestive tract from mouth to vent. Technically this propelling movement of the gut is called peristalsis, and depends for its steady rhythm, partly at least, on roughage. Fibre, cellulose and husks constitute roughage in the animal's diet.

To revert to water for a moment, it is becoming increasingly clear from the results of many experiments (Parkes *et al.*, 1946-50) that all types of laboratory animal require to be supplied with adequate amounts of water in their diet. This may either be given in the form of adequate amounts of fresh green-stuff or as a wet mash, or it may be supplied separately from some form of water container. Indeed, if a young mouse is to grow quickly and at a uniform rate, approximately 40% of its total intake of food should be water.

Completeness of diet does not, however, consist only of correct bricks, mortar, calories, water and roughage. Cathcart (1928), in answer to a question concerning a good diet for human subjects, replied: "The diet must (*a*) be sufficient in quantity to cover energy requirements (i.e. calories); (*b*) be of correct quality to satisfy biological needs (i.e. complete as regards essential amino acids); (*c*) be appetizing, that is, it must stimulate those reflexes which liberate digestive juices and enzymes, and it must promote peristalsis; (*d*) conform, as nearly as possible, to *racial* dietary habits. This relates nutrition to genetics and is the subject of much intensive research work throughout the world." Cathcart's words apply with equal force to diets of animals bred and intended for use in laboratory experiments. Some of the finer details applicable to each of these general principles vary according to the species of animal we are considering. Others as yet are matters of opinion rather than of rigid regulation. Nevertheless, the broad principles of animal nutrition are well established, and those in charge of animal stocks should study and be guided by them when planning to fill their storehouses with the raw materials needed for the maintenance of their breeding colonies.

(E) Putting Together a Complete Diet

The problem of feeding animal herds, stocks or small colonies is many-sided and involves nutritional, economic and local factors. The nutritional factor has been briefly surveyed in this article; the economic factor in animal husbandry cannot be entirely divorced from the scientific, nor can it be separated (when thinking of the commercial breeder) from local factors. These include his personal skill, space,

growing-ground, and apparatus (storage bins, racks and feeding pots, to name only a few items). For the man endowed with ten talents of skill, five acres of good kitchen garden, a dozen good metal bins and a large airy shed, the problem is easier (i.e. different) than for his less-fortunate competitor with one talent, ten perch of garden, two small bins, a few boxes and a tiny hut for workshop. There are, however, certain basic principles applicable to both breeders; we will imagine a compromise (in talents, space and apparatus), and proceed as if we are planning to make a complete diet for an allover colony of about 300-500 guinea-pigs, which includes breeding sows, boars, weanlings and growing young stock.

Our breeder favours the use of a home-mixed diet rather than a manufacturer's cubed diet. He therefore decides on the composition and quantity of his daily feed, buys the necessary ingredients to last him for an agreed period of time (e.g. 3 months) and stores them suitably. He has the apparatus and space for weighing and measuring the ingredients, for mixing, and finally for storing the finished diet. Diet 18 (Bruce and Parkes, 1947) may be used as the basis of our imaginary mix.

Bran	15
Barley Meal	20
Ground-nut cake	15
Linseed cake	10
Dried meat and bone meal	8
Dried grass meal	30
Calcium carbonate	1
Sodium chloride	1
	100

Before putting together these raw materials to form a diet, let us reassure ourselves that there is sound evidence for our faith in the finished product. A simple, although incomplete, basis of this belief is the analysis of the house of diet into the bricks with which we are now familiar. So here is the chemical analysis of Diet 18, quoted from the same source as its formula.

(*a*) Crude digestible protein	16·5
(*b*) Fat	4·6
(*c*) Soluble carbohydrate	33·7
(*d*) Fibre	6·7

Note that (*c*):(*a*)::2:1

The pattern of this analysis, we can rest assured, is in conformity with the accepted opinion of what is understood by a complete diet.

The utensils needed for making our mix are a table or bench, a tray with sides, scales and weights and/or measures (e.g. jugs, small bins) scoops, spoons, and—a pair of clean hands. The quantities given in the above formula add up to 100. All the ingredients are solids. In stating medicinal formulae, e.g. those given in statutory books of reference like the *British Pharmacopoeia*, *The British Pharmaceutical Codex* and *The British Veterinary Codex*, there is a rule when reading a formula which reads: "Liquid, by measure, solids by weight." In our fomula, we have only solid ingredients, and so the quantities are in grams, ounces or hundredweights, according to how much diet we are proposing to make and store.

There is one safe rule in making diets and that is to start with the smallest quantities and gradually to incorporate the ingredients of larger quantities. Thus in our formula we shall start by weighing and mixing together the two last-named ingredients, calcium carbonate and sodium chloride.

One other consideration must, however, be our guide. A light ingredient such as bran is more difficult to incorporate uniformly in a mix than is a heavier one like ground-nut cake. It is therefore advisable first to mix bran and ground-nut cake together separately before adding this mixture bit by bit to the already mixed salts. The addition of the remaining components then follows, stirring or manipulating the mix thoroughly, between each addition of an ingredient, as well as at the end of the whole process. When the finished product has been transferred to its storage-bin, a label or distinctive mark should be fixed to the container. This is then closed and stored in a cool place. Those who use a large sanitary dustbin 24 in. high by 18 in. diameter, as their storehouse, may like to know that its capacity, as regards feeding-stuffs of the texture of Diet 18, is about 40 kilos or 90 pounds.

Up to the present, this article has been concerned with problems of complete diets, their components and, on a small scale, the making of powdered diets to ensure their uniformity. The breeder, however, may not primarily be interested in powdered diets, but in mixed feeds and possibly in cubed or pelleted diets. Everyone realizes that fashions abound in human feeding as shown by Drummond and Wilbraham (1939) in their book *The Englishman's Food*. Similarly, the small animal stock-breeder has his dietary preferences, and anyone who has visited the caviaries of conscientious breeders will be reluctant to criticize

well-established customs. Nevertheless, there arises in almost everyone's lifetime occasions demanding a modification in habit, or a call for a change of routine, as prescribed by advances in knowledge. Hence, it is proposed to consider these departures from the norm under the next heading.

(F) Special Diets (and Field Trials)

"Field" in the vocabulary of the small animal breeder means colony trials—indoors rather than in the large stock-breeders' open fields and pastures. The principles underlying such trials, however, are very similar. As to special diets, the types envisaged are *not* deficient diets, nor those restricted deliberately by withholding adequate bricks from the edifice. Any such diets come within the definition of animal experimentation over which the Home Office has control, and hence for the commercial breeder of laboratory animals are out of bounds.

Special Diets for the breeder of laboratory species connote adequate complete diets; they come within the legitimate scope of a breeder who wishes to ring the changes in feeding-stuff for his animal colony. Four different situations spring to mind. (*a*) Breeder A wishes to try a pelleted diet for his cavies, instead of his present mixed food. (*b*) Breeder B has been troubled with occasional still-births, emaciated weanlings and the whole colony slightly below par, and has been advised to use either poultry pellets, or brewer's rye residues or yeast as a (1 in 10) supplement to his normal food. (*c*) C has just moved into a small-holding and therefore proposes to use homegrown green food and roots instead of buying them. (*d*) D has changed his source of supply of hay.

How can A, B, C and D plan a field trial with safety and on sound scientific principles and collect results which will lead them to justifiable conclusions! The safety slogan is: Always run a Control Group of Animals. Each of the above examples, in its appropriate context, calls for a small but representative group of animals to be fed on the special trial diet, and another group of animals, fed on the normal colony diet. What shall be the criteria? (*a*) growth, (*b*) absence of casualties and sickly stock, (*c*) average litter size and weaning weight, (*d*) breeding performance of one or more subsequent generations, (*e*) appearance, liveliness, and firmness of muscles, etc. Every experienced breeder has his own standards of judgment.

The colony trials on special diets or on proposed modifications thus call for careful planning, accurate records, comparisons and assessment

of results. Based on these fundamental principles, coupled with experienced observations, such trials will yield convincing objective answers and not mere subjective results and may make a welcome contribution to the common coffer of knowledge of small animal husbandry.

(G) Weights and Measures

In the past many successful breeders compounded the food their animals required without the aid of any other measuring or weighing equipment than simple containers and domestic kitchen tools. How often has one heard "three bucketfuls of crushed oats, one bucketful of bran, two pint mugs of this and a tablespoonful of that"?

Times have changed, and there is a demand for highly uniform animals in the modern laboratory. How can this be achieved if a casual method of diet preparation is used? It is far better to weigh out the amounts of the required ingredients on suitable scales and use suitably marked measures of volume that are uniform from one establishment to another. The very components of the diets often vary from batch to batch introducing differences, and these differences may well be considerably magnified if a volumetric method be used (for example a fairly uniform material like wheat can vary between 50 and 70 lb to the bushel).

The metric system is usually used in laboratories today for weights and volumes while the quantities of the ingredients in a diet are often expressed as a percentage, so the two systems are very easy to use and involve much less arithmetic than if one was using weights, for example, in pounds and ounces. Suitable scales are readily available and the price is not so much as to rule them out on grounds of economy.

(H) The Storage of Diets

This aspect of animal husbandry is very important, and its seriousness is not always realized by the breeder or laboratory animal worker. Good storage involves protection from (*a*) living marauders, such as flies, cats, dogs, vermin, mites, etc., all of which are likely carriers of infection; (*b*) damp; (*c*) heat; (*d*) light; and (*e*) excessive oxygen (remember that some vitamins are readily oxidized, especially vitamin C).

Almost all the evidence so far supports the use of covered metal bins, and puts sacks, paper bags and open wooden boxes out of court. Although there is some evidence from users of some forms of cubed

diet that the use of a full metal bin (with airtight lid) causes the lower layers of cubes to sweat, this technical hitch does not disprove other evidence which upholds the claims of the metal bin against its rivals.

(I) Keeping Records

Book-keeping is a universal art, but its pattern varies with individuals. Some will keep neat, double entry columns, whilst others prefer loose-leaf books, or a card index. Not a few prefer the old-fashioned skewer! Similarly there are different ways of keeping breeding and progress cards. A few general principles must suffice to guide the breeder who wishes to improve his system.

First of all he requires a book with an index to pages, or a card index. This should be kept in a safe place. Records should include the following information.

(*a*) A number for the parents.
(*b*) A number for the offspring.
(*c*) Dates of births, weaning, etc.
(*d*) Numbers in litter.
(*e*) Their weights at weaning (plus date).
(*f*) Casualties.
(*g*) Notes on diet changes if any, or unpredicted happenings (extra cold weather, lack of cabbage, etc.).

If cards are used, it may be advisable to have more than one colour, using one for parent stock, a second for animals weaned and a third for their destination (user's name).

Simplicity is the keynote of success, and a scheme that suits one's temperament, has been tried out and found reliable is better than the theorist's far-off dream. As for records, like babies and toothbrushes, everyone prefers his own. But he ought to have one—toothbrush and record, I mean!

Interesting systems of recording for breeding colonies have been described by Bruce and Parkes, and also by McKinlay.

References

Bruce, H. M. and Parkes, A. S. (1947). *J. Hyg., Camb.* **45**, 3, p. 328.
Bruce, H. M. and Parkes, A. S. (1947). *J. Hyg., Camb.* **45**, 70. (Quoted from Table I.)
Cathcart, E. P. (1928). "Nutrition and Dietetics." E. Behn: London.
Drummond and Wilbraham (1939). "The Englishman's Food." Cape: London.
McKinlay, H. (1950). *J. Anim. Tech. Ass.* **2**, 2, p. 2.
Parkes, A. S. *et al.* (1946-50). *J. Hyg., Camb.* **44-8** (I-XII).

2. THE NECESSITY FOR AN EVENLY BALANCED DIET

G. Porter

The laboratory animal living in captivity is deprived of access to its natural food, therefore we must overcome this artificially by providing an evenly balanced diet, equivalent in composition to the food stuffs normally eaten by the animal in its natural habitat.

The composition of an evenly balanced diet required by one species will differ from that required by an entirely different species; the basic requirements, however, remain constant, i.e. moisture, proteins, carbohydrates, fats, vitamins and minerals in adequate and balanced proportions.

(A) Moisture

I know of few mammals that do not drink water even where the diet consists of a wet mash and green food. Climatic conditions affect the moisture content of natural foodstuffs and also influence the water requirements of the animal, which acquires a certain amount of moisture from the food it consumes and from the oxidation of proteins and fats within the body. This is insufficient, however, to meet physiological needs.

A comprehensive guide to the amount of water consumed by laboratory animals cannot be given as there is a considerable variation within each species. Bruce (1950) draws attention to the rapid rise in the amount of water drunk during lactation. She found that the laboratory rat during lactation required three to four times as much food and water as was required for maintenance, therefore it is impossible to do justice to an animal during lactation unless fresh drinking water is supplied.

Many excellent animals are produced without a supply of drinking water, but only when the animals are provided with succulents. The commercial breeder is reluctant to adopt an expensive and laborious method of supplying drinking water. Tuffery does, however, point out (p. 36) that it is less laborious than mixing mashes, therefore an adequate supply of drinking water should be available to the animals at all times.

(B) Protein

The term protein does not signify an individual compound but it is the class name given to a group of substances. In fact there are many

proteins that may have identically the same percentage of carbon, hydrogen, oxygen, nitrogen and sulphur, yet differ from each other chemically and physically. As proteins are found in abundance in soft living tissue, they are essential in a diet to build healthy tissue and to replace that lost in normal physiological functions.

Before proteins can perform this function they must, in the normal course of digestion, undergo a chemical change into many separate simple substances (amino acids). These substances are then assimilated and circulated in the blood stream to all parts of the body where the cells select particular amino acids essential for their normal functioning. As there are many amino acids, any one type of protein may be lacking in one or other of those essential to the complete diet. One type of protein should never, therefore, constitute a diet, as, irrespective of the amount eaten, the animals may fail to grow and reproduce. Many of the balanced diets used by experimental units appear to be complex and have a great variety of constituents. This is not a nutritionist's fad, but rather a method of ensuring that the animals receive all the essential substances necessary for supporting normal growth and reproduction.

Digestibility must be considered when deciding to feed any particular source of protein, as an indigestible substance with a high protein level is of less practical value than a digestible substance of low protein level.

(C) Carbohydrates

Carbohydrates may be termed the energy fuel of the living animal and are found in various cereals, vegetables and animal by-products. Approximately 60% of this energy fuel used in the form of animal sugar is stored in the liver and muscles in the form of glycogen or animal starch; this store provides the amount of material necessary to keep the sugar in the blood at a remarkably constant level until further food has been eaten and digested. In this way the tissues have access to a fairly constant supply of sugar to provide heat and body energy, and any surplus after all requirements have been met may be stored in the form of fat.

(D) Fats

There is a wide variety of fats in nature. The function of fats is to act as a reserve force of heat and body energy when the diet is temporarily low in calorific value; for its own sake it is necessary in

performing certain functions essential to health and well-being. A number of vitamins are soluble in fat and some of the fats used in animal feeding are in themselves important sources of vitamins. The relationship of fats to other dietary constituents is a highly scientific subject and cannot be dealt with here. It may be considered that the essential fats necessary for good animal nutrition will be found in most of the recommended diets fed under normal conditions.

(E) Vitamins

Today nearly all vitamins have been isolated, purified, and many even produced synthetically in the laboratories. This makes it possible for a much closer study to be made on the part they play in the fundamental but obscure process of life.

1. vitamin A

At least five substances present in natural foodstuffs can supply vitamin A. Four of these are yellow pigments (the carotenes) soluble in fat, but hardly soluble at all in water, and a wide distribution of the carotenes is found in all yellow and green vegetables. The degree of colouring is a rough indication of the richness of the plant in carotenes. In contrast, vitamin A itself is not highly coloured and is found in its pure form in different kinds of fish liver oils. Vitamin A has been called the anti-infection vitamin and is necessary for normal growth, reproduction and lactation. The absence of this vitamin in the diet will affect the reproductive organs of the female, may prolong gestation and result in difficult parturition with possible death of the dam or foetus.

2. vitamin B complex

Vitamin B complex is a group of several substances classed together because they are water soluble, yet each individual vitamin in this group has its own particular physiological function to perform, each one being an essential dietary substance. Therefore the absence in the diet of any one item of the group will produce a deficiency. Adequate supplies of this group will be found in liver, whole milk, whole cereals, vegetables and brewers' yeast. These feeding-stuffs are incorporated in most of the cubed and pelleted animal foods.

3. vitamin C

The vitamin C requirement of guinea-pigs is of great importance, therefore, special mention of it should be made. The daily requirement given by Coward is 2 mg, yet Bruce and Parkes (1947) found that a

greater amount was necessary and again Dunn and Cowgill (1953) state that the vitamin C requirements of the guinea-pig are in direct proportion to its body weight. When considering how to supply the guinea-pigs with vitamin C, the only practical method is to feed adequate palatable green food. Lane-Petter (private communication) states: "A guinea-pig colony showed signs of scurvy when the diet contained large quantities of vitamin C given in the form of savoy cabbage. These scorbutic animals were in fact refusing vitamin C as it was being offered in an unpalatable form."

4. VITAMIN D

This vitamin regulates the metabolism of calcium and phosphorus in the body, and is thus concerned in the proper formation of bones and teeth. In discussing the dietary requirements for vitamin D it is always necessary to bear in mind that exposure of the body to direct rays of the sun may serve the same purpose as the ingestion of vitamin D in the form of food materials. Because of the relationship of vitamin D to calcium and phosphorus metabolism, it would be natural to expect the need for it to be most evident at that period of life when the formation of bone from the calcium and phosphorus of the food is in most active progress, that is in the young animal where a characteristic of vitamin D deficiency disease is rickets. Vitamin D is obtained either from fish liver oils or is produced in the skin by direct sunlight.

5. VITAMIN E

The action of vitamin E is probably widely diffused throughout the body. With the exception of the goat, this vitamin is essential for reproduction, and its absence will produce irreversible sterility in the males of many species.

(F) MINERALS

Minerals are necessary for general health as they help to regulate water concentration in the body, influence gland secretion and keep blood and tissue fluid from becoming either too acid or too alkaline.

1. CALCIUM

Calcium is the outstanding element in the mineral matter, giving shape and permanence to the body framework. It endows the bones with the strength and the teeth with the hardness that they need. There is a serious calcium problem in animal nutrition, partly because the calcium compounds which are abundant in nature are relatively

insoluble, and partly due to inadequate amounts being included in the diet to meet the added requirements of pregnancy and lactation.

2. PHOSPHORUS

While the amount of phosphorus in the body is not so large as that of calcium, a very large quantity and proportion of the phosphorus belongs to the more active tissues, and so, as would be expected, there is a more rapid turnover of body phosphorus than of body calcium. The ratio of needed calcium to needed phosphorus is perpetually changing with the development of the body, and in the later stages of pregnancy and in lactation it is again different from that of adult maintenance. Full discussion of this fact would be too long and complicated for this paper.

3. IRON

The study of iron metabolism has been closely related to the study of nutritional anaemia and haemoglobin formation and regeneration. Iron regulates the concentration of oxygen and carbon dioxide in the blood stream. The proportion of iron in the diet that is absorbed and can be used depends partly upon the foodstuffs eaten.

(G) IODINE

Iodine is an essential constituent of the thyroid gland; it is necessary for the formation of thyroxin which regulates the basal energy metabolism and growth.

The vitamin and mineral requirements of any given species cannot be dealt with on a general basis, as that of each vitamin and mineral is in itself a highly scientific subject. Coward deals with the vitamin requirements of laboratory animals. A comprehensive list of "Foodstuffs commonly used in stock diets and the vitamins they contain" by Farquhar (1953) is reproduced in Table I. Dyer (1951) outlined the vitamins, their source of supply and stability and the effect of a deficiency. The information given by these workers is of the highest value to the animal breeder, and the brief description of vitamins and minerals given in this paper should only be treated as a re-introduction to their highly informative papers.

Each component of an evenly balanced diet has its own particular function to perform. If any of these dietary essentials are lacking or not present in balanced proportion, reproduction, lactation and growth

TABLE I

Type of foodstuff	*Vitamins*	*Excellent*	*Good*
Animal Product	A, D	Fish liver oils, lard	
	A, B	Liver	Whole Milk
Vegetables	A, B, C	Kale Spinach Water Cress Carrots	Brussels Sprouts Turnips
Cereals	B, E	Wheat, Corn and Rye germ Whole Wheat Oats Soya Beans Peanut meal Bran	
Micro-organisms	B	Brewers' Yeast	

will be considerably upset; the full effects may take several generations to develop. A dietary deficiency can present a very complicated picture as the early symptoms of many deficiencies can look alike, the animal becoming susceptible to infection due to a lack of protective food. Unborn young may be considered as parasites and will acquire all the nutrients necessary, to the detriment of the dam. After parturition the position is reversed, sub-normal lactation will affect the litter. A large percentage of deaths among young animals is undoubtedly due to failure of the dam to lactate, the direct result of poor feeding. No attempt is made to influence breeders to use any particular diet, as that must, undoubtedly, remain a personal choice, to a certain extent controlled by experience, the species and strain, environmental conditions, and many other factors.

The large breeding establishments after many years of experience have found diets adequate to support oestrus, gestation, lactation and normal growth.

(H) SUMMARY

In the production of first-class animals the relationship between

good nutrition and selective breeding is a recognized fact. To gain full benefit from one, the other has to be clearly understood and both must be applied to the fullest extent. This paper, in layman's language, has attempted to outline the various factors and functions of the complex composition of an evenly balanced diet and how important the diet is to the health and well-being of a colony.

References

Bruce, H. M. (1950). *J. Anim. Tech. Ass.* **3**, 2.
Bruce, H. M. and Parkes, A. S. (1947). *J. Hyg., Camb.* **45**, 70.
Dunn, M. and Cowgill, G. R. (1953). *J. Nutr.* **9**, 507.
Dyer, F. J. (1951). Laboratory Animals Bulletin No. 2; 10.
Farquhar, Vivienne (1953). *J. Anim. Tech. Ass.* **4**, 14.

3. DIETARY VITAMIN C AND ROUTINE CARE FOR GUINEA-PIGS

D. Brown, I. D. Ferguson and A. G. Ramsey

The satisfactory breeding and rearing of any animal depends on an adequate diet, both in quality and in quantity. It must be stressed that it is vital that an adequate amount of vitamin C be given in the diet of guinea-pigs *every* day, even throughout the winter. Unless guinea-pigs receive adequate vitamin C every day, the breeder will have a low rate of reproduction in his colony, a slow growth rate and a heavy loss of guinea-pigs due to infection.

(A) Functions of the Diet

The diet is used by the animal for growth, to supply the body with energy, to synthesize internal secretions and also to supply vitamins, inorganic salts and water. The vitamins are accessory food substances which are essential for the health of animals. Only minute quantities are required in the diet, but if they are absent then animals no longer breed and grow; indeed they may become diseased and die.

(B) Vitamin C for Guinea-Pigs

The guinea-pig is different from mice and rabbits since it is unable to manufacture any of its own vitamin C. Consequently, if the guinea-pig does not receive vitamin C in its diet every day it will suffer from a lack of this necessary factor and develop avitaminosis C or scurvy. Provided that they receive an adequate supply of vitamin C, guinea-pigs can be

maintained in a healthy condition in which they breed well and the young grow rapidly.

This supply is readily available for guinea-pigs in fresh foods such as the leaves of green vegetables, for example raw cabbage leaves, lettuce, cauliflower, broccoli and kale. Vitamin C is also naturally distributed in other fresh foods, especially in the juice of citrus fruits, such as lemons, and in tomatoes and black currants. However, these alternative sources of vitamin C are more expensive and less easily available and are not used in feeding guinea-pigs.

(C) Synthetic Vitamin C for Guinea-Pigs

Vitamin C was first isolated from fresh foods in 1928 and was given he name ascorbic acid. It was not until 1933, however, that it could be artificially synthesized. It is now available commercially and we have found it possible to maintain colonies of guinea-pigs for at least a year on a diet completely lacking in natural vitamin C but supplemented by ascorbic acid which had been prepared commercially. Such a procedure is impracticable for routine maintenance guinea-pig breeding. It necessitates, in addition to the routine cage cleaning and supply of food and water, that a measured quantity of the artificial vitamin be dissolved in a known small amount of water and then be given in measured volume by mouth from a teat-ended pipette to each guinea-pig in the colony. If this tedious procedure is followed, it should be carried out every day. The commercial vitamin C is expensive to purchase.

(D) Routine Laboratory Conditions for Guinea-Pigs

For mass breeding from a high-grade stock, we use the polygamous system, since it is unnecessary to use paired litter mates of known age in most laboratory experiments. The pregnant sows are kept in the colony and the young reared without obvious bad effects.

Guinea-pigs maintained inside a laboratory are always kept under the best conditions which can be achieved. Two reasons for this high standard are obvious. The first is that inspectors appointed by the Home Office visit, from time to time, all places where experiments on animals are being carried out. The second reason is that if guinea-pigs are used in some form of biological assay, using that term in its widest sense, then it is essential that different guinea-pigs from the same colony give approximately the same uniform response to the same stimulus. A few examples of widely differing uses in which uniform

guinea-pigs are required include the diagnosis of human tuberculosis, the testing of new drugs for their biological potency of chemical substances present in amounts too minute to be detected by sensitive chemical tests, and in the training of medical students.

Since many laboratories desire to place guinea-pigs on experiment as soon as possible after their period of isolation following purchase, it is not unnatural that they will prefer to purchase guinea-pigs from a breeder whom they know from experience maintains, like themselves, a uniform and high standard of care of guinea-pig colonies. The essential is to fix a certain standard and then to maintain it.

1. TEMPERATURE

It is more important to maintain a constant environmental temperature over a relatively small range than it is to worry about the absolute values of the small temperature variation. In practice, the actual values which we could maintain conveniently were from 64° to 69°F. Any considerable temperature fluctuations will inevitably act as a non-specific form of stress, causing a fall in the normal growth curve of the guinea-pigs and decreasing the number of matings.

Short (1951) gives some advice on housing guinea-pigs in laboratories. Among his recommendations he mentions that a thermostat connected with the heating apparatus will ensure constant heat at any desired temperature. Breeders would be well advised to install this relatively inexpensive aid, thus maintaining better guinea-pig colonies.

2. PENS AND BEDDING

For bedding use clean sawdust with a liberal covering of oat straw to be renewed thrice weekly when the pens are cleaned. The straw provides an ideal source of roughage in the guinea-pig's diet. It is known that guinea-pigs require more bulk in their diet than rabbits and fail to thrive on a diet containing only 3% cellulose.

3. WATER

Fresh green food is unable to meet a guinea-pig's requirement for water if the animal is maintained on a diet of dry pellets. If open pots, troughs or drinking fountains of water are used for guinea-pigs, the water will inevitably become fouled with particles carried by the animals' feet and by urine and faeces, thus readily spreading intestinal infection. Moreover, such water containers, unless constructed with a heavy base, are often upset and the contents spilt. For the routine care of

guinea-pigs we recommend the commonly used method of providing inverted glass containers of water, each with a glass spout projecting into the cage or pen. The spouts are attached to the containers by rubber stoppers. Alternatively practical details on how to attach the spouts to any type of bottle are given by Lane-Petter (1951). The bottles are refilled daily. It is much more convenient to have two complete sets, so that the empty bottles can be removed and immediately replaced by full ones. The empty bottles are then thoroughly cleaned of regurgitated food particles before being temporarily stored. It is important that both sets contain a sufficient number of bottles to ensure that there is water available to the guinea-pigs even before the bottles are due for refilling for the day. A small stock of spare glass containers and glass spouts is required.

4. PELLET FEEDING

We have constructed, from empty Dettol tins, large pellet hoppers on the same gravity feed principle as the M.R.C. pellet hopper for rabbits. Nevertheless, each hopper had to be modified slightly by a process of trial and error, until it is found in practice that the pellets neither spill out to waste nor stick in the gravity feed. The guinea-pigs feed from an opening in the hopper 2 in. from the floor and $1\frac{1}{4}$ in. broad. No contamination by urine and faeces occurs, since this space is inconveniently narrow for all but the youngest guinea-pigs to climb upon. Since the top of the hopper is 10 in. high it is necessary to close this with a lid, with the result that the hopper can very readily be refilled and is easy to clean. It is necessary, however, occasionally to remove the accumulation of fine fragments of the pellets from the feeding opening.

5. GREENS

Of all the procedures involved in the routine care of guinea-pigs, this is the part most liable to be omitted. Again the use of a gravity food container is the easiest. A basket, size 10 in. by 10 in. by 1 in. is constructed from 1 in. hexagonal wire mesh and suspended so that the bottom of the basket is about 6 in. from the floor. It is vital that only fresh greens be given and that any dried greens remaining in the basket be removed daily. Since the greens are not fouled by excreta or even trampled upon by the guinea-pigs, this is not difficult. It is known that contamination of greens by excreta leads to oxidation and destruction of the ascorbic acid content.

(E) VITAMIN C REQUIREMENTS OF GUINEA-PIGS

The quantity of vitamin C which a guinea-pig requires for maximal growth and reproduction depends upon a very large number of factors e.g. age, sex, whether pregnant or not, whether suckling or not, the route by which the vitamin C is obtained, e.g. in fresh green-stuffs intermittently throughout the 24 hours, or as one dose of synthetic ascorbid acid.

Bruce and Parkes (1946) drew a distinction between green food as a vehicle for water and green food as an essential source of vitamins. Subsequently Bruce (1950) put forward some of the details of experiments which she interpreted as showing that green food given in excess of 30 g per day to guinea-pigs is used only as moisture and could better and more cheaply be given in the form of water. Making a gross estimation of the ascorbic acid content of greens as 60 mg ascorbic acid per 100 g greens, this means a daily intake of the order of 18 mg vitamin C per guinea-pig per day.[1]

Our experiments on the vitamin C requirements of guinea-pigs carried out during the last 5 years indicate that cabbage is better for guinea-pigs than an equivalent amount of synthetic ascorbic acid, or in other words that cabbage is dietetically more than a mere vehicle for ascorbic acid. It may be that other factors present in the cabbage are important for guinea-pigs. Our present opinion is that guinea-pigs, no matter their weight, age or sex, should be allowed 80 g of fresh green food per head per day which would contain approximately 45 mg vitamin C, probably a closer estimate of the requirement of vitamin C by the guinea-pig. This is in addition to unlimited amounts of straw, water and about 60 g of pelleted diet per head per day. Using this regimen under the routine conditions given, guinea-pigs have a calculated mean period from the probable first oestrus to fertile mating of only 2 days. Deaths of females during pregnancy are extremely uncommon, as are still-births. The litters are larger in numbers, an average of five per litter being weaned, with only three litters being taken annually from each sow. The young, as well as being uniformly heavier at birth, grow more rapidly and regularly. Infection is practically unknown and obvious ill-health due to a virus pneumonitis, probably inherent in all guinea-pigs, is no longer seen (Brown *et al.*, 1952).

[1] The requirements of the guinea-pig for vitamin C are not known with certainty and appear to vary with the type of husbandry employed. This explains discrepancies that will be found between statements by various authors.

(F) Summary

The adoption of certain optimal conditions for breeding and rearing guinea-pigs is justified by the results obtained. The value of a regular routine is stressed, particularly the necessity for the adequate provision daily of fresh green foods containing vitamin C. In spite of the long gestation period, breeders can thus supply laboratory demands more rapidly and provide healthier guinea-pigs which show less scatter in age/weight relationship and prove more satisfactory for biological assays. Breeders can even supply paired litter mates upon special request, due to the larger numbers in each litter and to the much smaller losses. Finally it is less necessary to select the herd since there are few bad breeding records.

References

Brown, D., Ferguson, I. D. and Ramsay, A. G. (1952). *Brit. J. Nutr.* **6**, 3. "Guinea-pig growth and reproduction on cabbage or on synthetic ascorbic acid."

Bruce, H. M. (1950). *J. Anim. Tech. Ass.* **1**, 3, p. 2. "The water requirement of laboratory animals."

Bruce, H. M. and Parkes, A. S. (1946). *J. Hyg., Camb.* **44**, 501. "Feeding and breeding of laboratory animals, II."

Ferguson, I. D. and Ramsey, A. G. (1953). *J. Physiol.* **121**, 37. "An automatic refilling water container for laboratory animals."

Lane-Petter, W. (1951). *J. Anim. Tech. Ass.* **2**, 3, p. 14. "A soft plastic cap for water bottles."

Short, D. J. (1951). *J. Anim. Tech. Ass.* **2**, 3. "Cavies. A short history of some useful breeds and their general management."

4. NOTES ON GREEN FOOD FOR GUINEA-PIGS

G. Porter

Vitamin C in the form of green food is one of the necessary items of a guinea-pig's diet, without which a scorbutic condition would develop, thereby reducing the level of resistance to infection. In contrast to popular belief, the guinea-pig is quite fastidious regarding green food and by choice will never eat savoy cabbage, the tops of carrots, swedes, turnips, radishes, parsnips or celery. Therefore in the selection of green foods consideration should be given to their palatability. Many other factors affect the health of a colony but the choice of good, clean, non-poisonous, green food is one factor the breeder can control.

(A) Common Faults

1. CHANGE OF DIET

A sudden change of diet may upset the animals; in this green food is no exception, and where a seasonal change is anticipated it is desirable to introduce the new food gradually over a period of at least 7 days before the old food has expired, otherwise, the animals may gorge themselves with disastrous results.

2. HOT OR MOULDY GRASS

Grass which has become hot or mouldy is a possible cause of intestinal trouble. Damp grass stored in a heap will become hot and mouldy overnight and is quite unsuitable for feeding next day. If grass has to be retained overnight it should be spread out evenly over a clean floor, away from possible contamination by domestic animals and poultry and not in a position to be trampled on.

3. GREENGROCER'S WASTE

The city breeder may use greengrocer's waste, which is undoubtedly cheap but most undesirable due to the high incidence of infection it may introduce. Waste matter is often swept up from dirty floors, deposited in barrels in an outer yard where wild rodents, cats and dogs have every opportunity to soil and contaminate it. In warm weather fermentation and decomposition takes place in these barrels.

4. LAWN MOWINGS

Lawn mowings should be fresh and clean, generally grass obtained from this source is dry and fibrous, and of very little food value.

5. FROSTED GREENS

Green food which has been subjected to a heavy frost should be cut 24 hours before it is required, and allowed to thaw at normal room temperature, approximately 60°F.

6. POISONOUS PLANTS

Green food gathered in the hedgerow will undoubtedly contain a proportion of poisonous plants and weeds. Some may be virulent, others cumulative, but neither will produce ill-effects if fed in small proportions along with other green food. It is wiser, however, to avoid putting into the hutch anything which is of a doubtful character. Healthy well-fed animals will avoid deleterious plants, but it is never safe to trust to their instinct, especially if green food has been in short supply.

The poisonous plants most commonly encountered when gathering

wild green foods are hemlock, beaked parsley, sheep's or asses' parsley, all of which are at times mistaken for wild and hedge parsley; deadly nightshade, which grows in the form of a bush and produces dark purple flowers in June and purple berries in August; woody nightshade, which has small greenish flowers and scarlet berries, commonly found in hedgerows and often described as deadly nightshade; foxglove, which cannot be mistaken, as it grows very tall with purple or white tubular flowers and a soft silvery leaf; water dropwort, which is not unlike wild celery and very common, and grows in marshy land or on river banks; common and black bryony, two vine-like climbing plants, which produce scarlet berries and are found growing in woodlands and hedgerows; the leaves of all ornamental and evergreen trees and shrubs, also leaves of oak, hawthorn and buckthorn.

Buckwheat is a plant which is not recommended when in flower; it is easily distinguished by the mass of pink and white flowers, and is found growing abundantly round the edges of corn fields.

It is perhaps unnecessary to add potato haulm, lupin, dock, sorrel, tansy, or rhubarb leaves, for no breeder would knowingly feed these.

These notes may prove helpful to less experienced breeders. If it is possible to feed good mixed herbage, the health of the colony will undoubtedly improve. The variety of available green food is partly controlled by the season of the year, geographical position and climate, but the choice of palatable fresh green food is in the hands of the breeder.

7

Breeding

1. BREEDING PRACTICE AND THE IMPROVEMENT OF LABORATORY ANIMALS

D. S. Falconer[1]

All who breed animals seek to improve their stock by their methods of breeding, whether they breed livestock for human consumption, horses for racing or dogs for showing; and the producer of laboratory animals for scientific research naturally wants to do the same, because it not only gives him satisfaction to breed better animals but also adds to his reputation and increases his profits. In this article, suggestions about breeding practices are made which it is hoped will enable the breeder to improve his stocks successfully and with little or no extra effort. The suggestions are based on the scientific study of the laws of heredity, about which much research has been done and is still being done. But there is no need for the breeder himself to understand these laws in order to practise sound breeding methods.

The essence of good breeding practice is selection—that is simply breeding from the best individuals. Do not sell your best animals, they are much too valuable. To decide which are the best, however, is not always so easy, and it is here that the following suggestions should be of most use.

(A) Desirable Qualities

First we must decide what qualities we want to improve in the animals. The most important are obvious: they are (*a*) general health and vigour, (*b*) high productivity, (*c*) good mothering ability and (*d*) tameness and ease of handling. Other qualities must be left to the breeder's own judgment and to the laboratories using the animals making their wants known. But a word of warning must be given. Do not breed for fancy points, such as the shape of ears, length of tail or general appearance. These things will not improve the real value of the animals and the more attention you pay to them the less will you be able to pay to the points that really matter. Most breeders regard large size and rapid growth as desirable qualities in their animals. Up

[1] Agricultural Research Council Scientific Staff, Institute of Animal Genetics, Edinburgh.

to a point this is justified, but do not go too far. Experience has shown that too great a change in any character may lead to deterioration in other characters which matter more, such as fertility.

(B) Heredity and Environment

Having decided which are the characters we want to improve, the next step is to see how we can decide which are the best individual animals to breed from. The important fact to realize is that an individual may owe its good qualities to two causes: it may inherit them from its parents, or it may have had a better-than-average environment. Only if the good qualities are inherited from the parents will the individual pass them on to its own offspring. External influences due to a good environment are *not* passed on. Therefore the skill of selective breeding lies in judging how far the individual owes its good qualities to its inheritance and how far to mere luck in the conditions of its early life. An example will make this point clearer. The size of laboratory animals is very much influenced by the number of animals in the litter. Even when adult, members of large litters are generally smaller than members of small litters, simply because the mother's milk has to be shared by all the offspring. Members of small litters, therefore, get a better start in life. Therefore, if you want to increase size and have to choose between two animals of equal size (and of equal age), one of which was a member of a small litter and the other a member of a large litter, you will choose the one from the large litter. It had a poorer chance in early life, therefore its large size must be due to internal, hereditary causes.

In some characters the influence of heredity is strong and that of the environment weak. These characters are in consequence easily changed by selection, and you need pay less attention to them. Size, growth rate, bodily shape and probably tameness are of this sort. The more important characters such as vigour, fertility and milk production, however, show a stronger influence of the environment and are therefore less easily changed by selection. For these characters, therefore, it is especially important that your judgment should be based on "equal chances for all". This means in practice that the animals from which you are to choose should be treated alike as far as is practicable; they should be fed alike and housed alike, and no preferential treatment given.

Before proceeding further, two points about the working of heredity must be made clear. Firstly, as stated above, the effects of external

influences are not passed on to the offspring. If you give an animal extra rations and make a giant of it, its offspring will be no bigger than the normal, unless you also give them extra rations. This does not mean that good conditions of feeding and housing are not important; it means only that the improvement of the quality of the stock thus obtained lasts only as long as the good conditions continue. Secondly, the inheritance of an individual comes equally from both parents. Evenly the purely female qualities such as good mothering ability are inherited equally from the father and from the mother. But since there are fewer males than females in the breeding colony, the influence of any one male on the future generations is stronger than that of any one female. Therefore, the choice of males is, if anything, more important than the choice of females, and males should be chosen with the greatest possible care.

(C) Breeding Practice

Let us now consider how the general principles outlined above might be applied in practice. It is not possible to suggest a detailed system of breeding because circumstances differ too much. Each breeder must therefore adapt the general principles to fit his own particular circumstances. The following general plan should, however, be applicable under most circumstances.

1. SELECTION FOR PRODUCTIVITY AND MOTHERING ABILITY

When a breeding female has reared her first litter her value for productivity and mothering ability must be judged, and the decision made whether to keep her young for breeding or to sell them. If the number of young reared is well above the average, and they are well grown and healthy, the young should be set aside for breeding; that is, selected. If not, they should be sold. Just how much above the average in productivity and mothering ability the female should be for her young to be worth keeping depends entirely on the number of young animals that are needed to replace old females that have finished their economically productive life. On this point no general advice can be given: all that can be said is that only litters of the best possible mothers should be kept, and that it is, of course, uneconomical to set aside more animals than will be needed for replacements.

2. SELECTION FOR OTHER CHARACTERS

Having decided whether a litter is to be kept for breeding, the next step is to consider whether all of the litter should be used or only some

members of it. This is the point at which selection for other characters such as tameness or rapid growth can be made. It is therefore best to keep all members of the litter till they are of breeding age so that these other points can have time to develop. Any very undersized animals can, however, be discarded at weaning. The important characters in females are fertility and mothering ability, and these cannot be judged till the animals have bred. But the litter has already been selected for these characters on the basis of its mother's performance, and if any of the females are discarded some more from less desirable litters will have to be taken to fill their place. Therefore, all the females in the selected litters should be kept for breeding except any that show serious defects such as very poor growth or extreme savageness. Of the males in the selected litters only a few need be kept, since fewer males are needed for breeding than females. Therefore, in the first place, choose the males for breeding from the litters of the very best mothers, and within these litters pick the individual males which please you best in respect of any other desired characters such as tameness and rapid growth.

3. SELECTION FOR HEALTH AND VIGOUR

When the litters chosen for breeding are adult, their general health and vigour will be apparent. If any member of a litter has contracted a disease, or shows any sign of ill-health, it will probably be wise to discard the whole of that litter. If more than one member shows ill-health it will certainly be wise to discard the whole litter. This means that when litters are selected at weaning, allowance must be made for some being discarded later on the grounds of ill-health, and enough litters must be kept to make good this loss.

4. SPEED OF PROGRESS

The rate at which the stock is improved by selective breeding in the manner outlined above depends principally on two things. The first is the amount by which the selected animals exceed the average of their contemporaries. Apart from skill and care in selecting, this is beyond the breeder's control and depends on the number of replacements needed. The second factor controlling the speed of progress is the length of time between one generation and the next; and this is in the breeder's control. The quicker the turnover of breeding animals, the faster will the progress be. This does not mean that the older pairs should be killed off before they have finished their economically productive life. But it does mean that the offspring of old pairs should not

be used for breeding. If the stock is continually improving it is obvious that the offspring of the young animals will be more advanced than those of the older, the offspring of the older parents being outmoded by the offspring of the younger. It is most important therefore that breeding males should be discarded when their females are replaced. An old male may be perfectly capable of satisfactory breeding with a new young female, but if any progress is being made by selection he will be outmoded, and a younger male is likely to be better.

5. RECORD KEEPING AND THE USE OF LATER LITTERS

The foregoing suggestions are made on the assumption that no detailed records of the animals' performances will be kept. Record keeping adds to the labour, and with reasonable care in a well-ordered animal colony it should be possible to practise the breeding methods outlined above without keeping records. It cannot be denied, however, that a simple system of recording the important facts, such as the date of mating, the number of young born and weaned and the parents of the weaned litters, would be an advantage because it would make it possible to use for breeding more than one litter from the best mothers. The value of a female for production and mothering ability can be better judged from her average performance in two or three litters than from the first alone. It was simply the assumption of the absence of records that led to the recommendation to breed only from first litters. If, however, records are kept and the average performances of females with two or three litters are available, the young animals in the later litters of the best mothers should be used for breeding. Really outstanding mothers might contribute young for breeding from litters later than the third, but this should not be done often because of the delay to progress caused by breeding from outmoded parents. The keeping of records has the additional advantage that it enables the breeder to follow his progress in raising the average performance of his stock.

6. A PICTURE OF THE PROCESS

It may help the reader to understand how the breeding system recommended works if the following simplified picture is imagined. Suppose a breeder has a colony consisting of 400 breeding females, each of which is housed in a separate cage; and that, for the sake of illustration, the animals are mice with an average breeding life of 200 days. Then, on the average, two old females will be discarded every day, and two young females mated up to replace them. Suppose

now that the 400 cages are arranged in a row on one long shelf, and that they are arranged in order of age, with the youngest on the left and the oldest on the right. Each time an old female is discarded from the right-hand end of the row the cages are all pushed along to the right and the new young female is put in the vacant space on the left.

Now, at about one-quarter of the way along from the left the females are weaning their first litters. These young animals, when mature, are put back into the stream at the left-hand end as young breeding females. Thus the stream replenishes itself from a point about one-quarter the way down. The rest consist of older females whose job is to produce only young animals for sale until they have to be destroyed. Suppose now that the first litters of each female contain at weaning four young females. The four females of one litter will then replace four old females. You therefore can reject three out of every four weaned litters. This is the selection by means of which you raise the average level of performance at the left-hand end of the stream. This increase at the left gradually works its way down to the right, but while selection continues, the left-hand end is always at a higher average level than the right-hand end.

If records are kept and you breed from second litters as well as from first, you can discard the litters of seven out of every eight females; or if third litters are also used you can discard eleven out of every twelve. Thus your selection would be more intense and the level of the head of the stream would be raised faster. But if you use litters later than about the third they will be coming from parents which have already passed a good way down the stream and they are not likely to be as good as the earlier litters of younger parents nearer the head of the stream.

This picture is, of course, much simplified, but it does represent what is happening in a colony which is being improved by selective breeding and at the same time producing young animals for sale.

(D) Outbreeding and Inbreeding

Many laboratories use inbred animals. These are special stocks with particular qualities and have been inbred by continuous brother-sister mating for many years. Most of them are difficult to maintain because their fertility and general vigour are low. To make an inbred stock is even more difficult and results in a great wastage of animals in the early stages. It is therefore strongly recommended that the producer of laboratory animals for general use should avoid inbreeding, and should leave the production of inbred stocks to the specialist. To

avoid inbreeding it is not necessary to keep elaborate pedigrees. It will be enough simply to avoid mating brother to sister, or to half-sister. If more than one litter of any pair of parents is being used for breeding, the need for some record keeping is again obvious, because the mating of a male from one litter to a female from another litter of the same parents, or even of the same father, is to be avoided. Again, the mating of an animal to its parent is to be avoided because it is too close inbreeding, as well as for the reason given above, that the parent is outmoded. The avoidance of close inbreeding is the only advice about the choice of mates among the selected individuals that need be given.

(E) Colour

Nothing has yet been said about the colour of the coat. This is because the different coat colours are inherited in a very simple way and the environment has a negligible influence on them. Therefore, to change the colour of the coat presents a different and much simpler problem to the breeder. Most breeders will probably want their stock to be uniform in colour. There is no real scientific advantage in having the animals all of one colour, but it is undoubtedly more pleasing to breeder and user alike. Any desired colour can be fixed throughout the entire stock in a very few generations (usually no more than three), and once fixed, the stock will breed true for that colour ever after, provided no animals are introduced from outside the stock. But to fix the colour in the quickest way requires a knowledge of the laws of Mendel, and these would require too much space to explain here. Therefore, only some general suggestions can be given. If you want to fix a particular colour in the stock, the animals for mating should be chosen in the following order of preference. (*a*) Animals of the desired colour (*b*) which have at least one parent of the desired colour (*c*) which have a brother or sister of the desired colour. If two animals of the desired colour, when mated together, produce any offspring that are not of the desired colour, the whole family should, if possible, be discarded.

Albinism ("pink-eyed-white") is the easiest colour to fix, and is the most generally useful colour to have, particularly in mice. What to expect from different sorts of mating can be very simply stated. Two albinos mated together produce nothing but albino offspring: an albino mated to a non-albino produce either about half of the offspring albino or none of the offspring albino: two non-albinos, both of which

had one parent albino, when mated together produce about a quarter of the offspring albino.

These are, admittedly, rule-of-thumb methods, but, if followed, they will enable you to fix the colour of your stock fairly rapidly.

The experienced breeder will see in this article nothing more than what he regards as common sense. But it may give the inexperienced breeder confidence to know that the common sense is based on the scientific knowledge of the laws of heredity. Furthermore, much experience has shown that selective breeding does in fact work. Our present breeds of livestock illustrate the power of selective breeding to improve the quality of the stock. But perhaps the most striking evidence is seen in the great diversity shown by the different breeds of dogs when contrasted with the uniformity among cats in which breeding cannot be effectively controlled. It can therefore be confidently expected that the producer of laboratory animals can do much to improve his stock by selective breeding, and thereby add to his profit.

2. INBREEDING

D. S. Falconer[1]

An understanding of what inbreeding is, and of what it does, is important to animal breeders of all sorts. There is a high demand for inbred animals by laboratories, and so it may be of interest to breeders of laboratory animals, whether they go in for inbred animals or not, to have an explanation of what is the special value of inbred animals. But the subject is really more important to the ordinary breeder who maintains outbred stocks, because a misunderstanding of the consequences of inbreeding may lead to the ruin of a stock. So the purpose of this article is to explain the consequences and uses of inbreeding. I am afraid it will not all make easy reading.

(A) What is Inbreeding?

First, what is inbreeding? Many people speak of inbreeding "for" something: for weight, for fertility, etc. This is most misleading. You cannot inbreed "for" anything: you can just inbreed. But you can at the same time select for desirable characteristics, and inbreeding "for" something means a combination of inbreeding and selection. The

[1] Agricultural Research Council Scientific Staff, Institute of Animal Genetics, Edinburgh.

combination makes a very complex problem and the two processes are best dealt with separately. Selection was the subject of the previous article: here we shall deal only with inbreeding. Inbreeding, then, means the mating together of individuals which are related to each other by ancestry. There are different degrees of inbreeding (measured on a scale from 0 to 100%) which depend on how long the inbreeding has been going on. And there are different rates of inbreeding (so much per cent per generation) which depend on the closeness of the relationship between the mated individuals. The rate of inbreeding is often referred to as the "closeness" or "intensity" of inbreeding. The greatest rate of inbreeding is achieved by self-fertilization, but that is possible only in plants. In animals the greatest rate is by full-sib matings or by parent × offspring matings, when the offspring are always mated to the younger of the two parents. Less intense systems of inbreeding are the mating of double-first-cousins, single-first-cousins, etc., and by still more distant relationships for which there are no names. We shall return to the rate of inbreeding later.

(B) Consequences of Inbreeding

The effects of inbreeding must be studied on two levels: firstly, what we know from genetical theory to be the inevitable consequences of inbreeding, and secondly, what is actually observed to happen when a stock is being inbred. It must be confessed that the ladder connecting these two levels is a flimsy one missing many rungs, with the result that the observed effects of inbreeding are still far from properly understood by geneticists. We shall deal with these two levels in turn.

1. theoretical consequences

To explain the theoretical consequences of inbreeding in a short article is impossible without assuming a little knowledge of genetical ideas. Those who have not this knowledge will, I am afraid, find this section rather indigestible. All the consequences follow from one simple effect: inbreeding increases the number of genes that are homozygous and decreases the number that are heterozygous in the individuals of the inbred stock. To understand the result of this increase of homozygosis we must examine more closely what is usually called the "structure" of the inbred stock. Suppose you start from an outbred stock and start inbreeding: we shall consider brother × sister mating for the sake of simplicity. You start with a number of families which are represented by the top row of circles on the left-hand side of the Fig. 7. Only five families are shown: the rest are to be imagined.

You then mate brother × sister through a number of generations, of which five are shown in the diagram, and you end up with a number of "lines", with no cross-connections since the inbreeding started. No two lines have a common ancestor until you go back to the outbred stock. This is one way of inbreeding, which may be called the "parallel-line" system. But you may start with only one family of the original outbred stock; you would then make several brother × sister matings from this one family. In the next generation you again pick only one family and make several matings from it, and you end up with several families all belonging to one line. This method of inbreeding, which may be called the "single-line" system, is illustrated on the right of the diagram. Now these two systems of inbreeding have entirely different results: they both produce inbred stocks, but the inbred stocks they produce are quite different. Let us see how this happens.

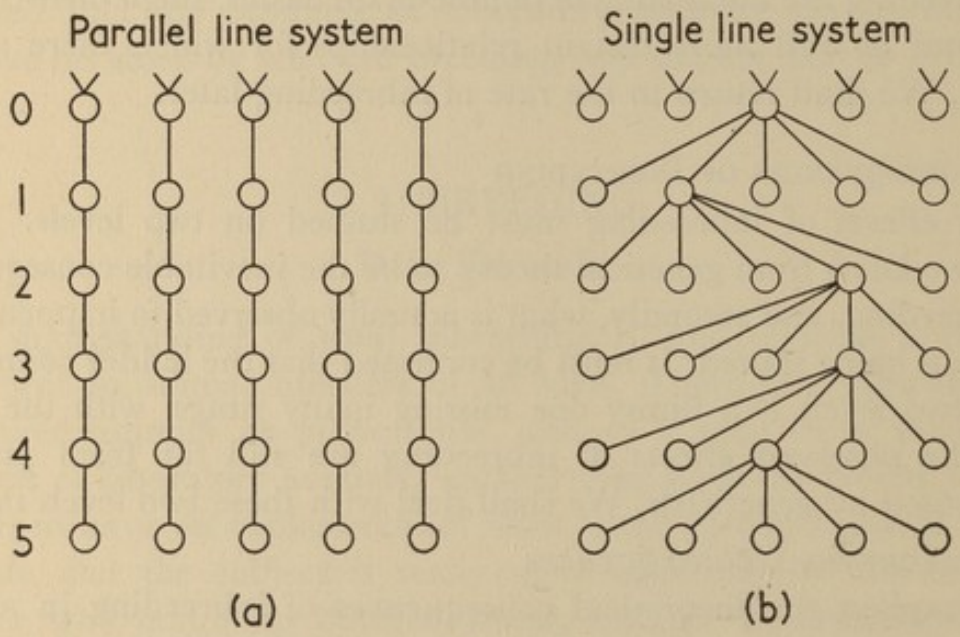


Fig. 7.

We return to the fact that inbreeding increases homozygosity. The result of this is that all individuals belonging to the same line tend to be homozygous for the same genes, but different lines are homozygous for different genes. Thus, inbreeding produces genetic uniformity within each line but genetic diversity between different lines. So an inbred stock produced by the parallel-line system contains much genetic or hereditary variation. In fact, its genetic variability is approximately doubled in the end after many generations of inbreeding. But an inbred stock produced by the single-line system contains little hereditary variation and is genetically uniform.

The parallel-line system may be used as an aid to selection. If you are trying to improve a character where there is very little hereditary variation but a great deal of non-heritable or environmental variation, it may sometimes be an advantage to increase the heritable variation by inbreeding in this way. The single-line system is used to produce genetically uniform strains for laboratory use, and the inbred animals demanded by laboratories are produced by this system. In the end, after many generations of inbreeding, all the genetic variation is eliminated. For practical purposes this state of affairs may be regarded as being reached after about twenty generations of continuous brother × sister mating.

There are two reasons why inbred animals are used by laboratories. First, variation among his animals is a nuisance to the experimenter because it reduces the precision of his results. The greater the variation, the more animals he has to use to attain a given standard of accuracy. Secondly, the experimenter wants successive batches of animals to be as alike as possible, in order to avoid discrepancies between different experiments. Uniformity within each batch and between successive batches is what the laboratory wants, and, therefore, inbred strains are used because they are known to be genetically uniform.

2. OBSERVED CONSEQUENCES

All that has been said so far is based on genetical theory and refers to the genetic situation in inbred stocks. We must now turn to the observed consequences and consider what actually happens when a stock is inbred. No attempt will be made here to connect the observed effects with the genetic changes which we know from theory must take place, because the connection is still incompletely understood.

Much the most important observed effect of inbreeding is the reduction of "vigour", and this is the only effect that need be discussed here. It is almost always found that inbreeding reduces fertility, mothering ability, growth rate and general health; all the characters, in fact, which really matter to the breeder. These harmful effects may sometimes be mitigated by rigorous selection during the inbreeding. But the situation should probably be viewed as a balance between opposing forces, the upward trend due to selection and the downward trend due to inbreeding, the inbreeding thus counteracting the good results of selection. Therefore, from the general breeder's point of view, inbreeding is to be avoided at all cost, whether accompanied by selection or not.

(C) Avoidance of Inbreeding

There are a few practical questions about how inbreeding is to be avoided which it may be helpful to discuss. To avoid mating brother × sister is easy enough even without the help of records. So we need not worry about close inbreeding because it is easily avoided. (But do not forget that two animals from different litters of the same two parents are just as much brother and sister as two from the same litter.) It is the slow inbreeding of which the breeder is unaware that needs care. Slow inbreeding arises in closed stocks in which after a time all the animals become distantly related to each other; a closed stock being one into which no outside blood is introduced by crosses with other stocks. If the stock is continually replenished by animals from outside sources, the problem does not arise and so the following remarks refer only to closed stocks. The rate of inbreeding in a closed stock depends on the size of the breeding population. By this I mean the number of parents whose offspring are used for breeding in the next generation. Obviously the breeding population, in this sense, may be very much smaller than the colony actually in production, because the offspring of many pairs may be sold and not used for breeding. So it is the number of parents whose offspring are used for breeding that matters. Let us see how large the breeding population must be in order to avoid serious inbreeding. There is a simple formula that tells us the rate of inbreeding with a given size of breeding population. This is $\frac{100}{8M}+\frac{100}{8F}$, where M is the number of male parents and F the number of female parents in the breeding population. The formula gives the percentage increase in each generation. For example, if the breeding stock came from six male and thirty female parents, the rate of inbreeding would be:

$$\frac{100}{8\times 6}+\frac{100}{8\times 30}=\frac{100}{48}+\frac{100}{240}=\text{(about) } 2+\tfrac{1}{2}=2\tfrac{1}{2}\%$$

Now the question arises: what is the highest rate of inbreeding that can be allowed? But there is no definite answer and I can only give my opinion: I should feel unhappy if the rate of inbreeding were over 2% per generation, and I should feel quite satisfied if it were under 0·5%. Let us see what this means in numbers of parents. The following table shows the rate of inbreeding with various numbers of male and female parents in the breeding population. The table probably covers the range likely to be met with in practice.

Breeding population		*Rate of inbreeding*
Male parents	*Female parents*	*% per generation*
10	10	$2\frac{1}{2}$
10	20	2
10	50	$1\frac{1}{2}$
20	20	$1\frac{1}{4}$
20	50	1
50	50	$\frac{1}{2}$

We see from the table that if there were only ten male parents we must have more than twenty female parents if the rate of inbreeding is not to exceed 2%; and we must have at least fifty parents of each sex if the rate is not to exceed 0·5%. As a general rule, therefore, I should take great care never to have fewer than ten male parents in the breeding population, and I should aim at having at least twenty of each sex, or even fifty if the total size of the colony permitted.

(D) Can Inbreeding be Undone?

If a stock has suffered a reduction of vigour through inbreeding, the inbreeding can be undone and the original vigour restored simply by outcrossing. This statement needs some explanation. If two stocks which have suffered a reduction by inbreeding are crossed, the cross-bred animals generally show an improvement of all characters lumped under the term vigour. This increase of vigour in the cross-bred stock is familiar to plant and animal breeders as hybrid vigour. The vigour is not always fully restored in the first cross-bred generation because some characters, such as early growth rate, depend on the mother: so their improvement does not appear till the second generation which has cross-bred mothers. Now we must consider what constitutes outcrossing. If a stock is inbred by the parallel-line system, outcrossing can be effected simply by crossing the different lines within the stock. The original outbred stock is thereby reconstituted. But a stock inbred by the single-line system is itself just one line, and it has to be outcrossed to a different stock if the inbreeding is to be undone. Now the important point for breeders is that a closed stock which has become inbred in the manner described above is a single line. The outbred condition, therefore, cannot be restored by crossing within the stock. Outcrossing must be made by crossing with a different stock, and the stock will therefore no longer be a closed one. So the

inbreeding in a closed stock cannot be undone while the stock remains closed.

(E) Inbreeding as an Aid to Selection

It was said earlier that the parallel-line system of inbreeding may be used as an aid to selection because it increases the hereditary variation. This needs some explanation because some breeders may be tempted to try it. You will remember that as the inbreeding progresses the hereditary variation comes to act more and more between lines and less and less within lines. That is to say, the lines become genetically different, but all members of one line become genetically alike. Therefore, selection must operate on whole lines and not on individuals within a line. It will be no good to pick the best individual from one line, because its superiority over other members of the same line will not be hereditary. Therefore, you must pick the best lines and discard the worst as wholes, using or discarding all individuals of the line. But here a difficulty arises, because if you go on discarding lines for long you will end up with only a few lines left. And the inbreeding then cannot be fully undone by crossing the lines.

As a general conclusion we see that inbreeding by the parallel-line system does no permanent harm so long as a large number of lines are retained to the end and then crossed. If some of the worst lines are weeded out on the way without seriously reducing the number of remaining lines, some permanent improvement may be gained after the crossing.

(F) Maintenance of Inbred Strains

The emphasis in this article has been on the maintenance of outbred stocks, how to avoid inbreeding, and how inbreeding may sometimes be used as an aid to selection. There may, however, be some breeders who maintain inbred strains for supplying laboratories, and so it may be useful to discuss a few of the problems peculiar to inbred strains. The making of an inbred strain is a specialist's job and will not be discussed: only the maintenance of already inbred strains will be considered.

The fact that inbreeding almost invariably results in a decline of vigour has been sufficiently stressed above. Most of the inbred strains used by laboratories are completely inbred: that is, the degree of inbreeding is 100%. Therefore, they suffer from the effects of this decline of vigour and are usually difficult to maintain. There is no way

of overcoming this difficulty except by giving the animals the best possible conditions. The problems to be discussed concern the system of mating that must be followed. The maintenance of an inbred strain is a most responsible job because the continued existence of the strain depends on the breeder. If a wrong mating is made and goes undetected, the strain may be completely destroyed and years of laborious work undone at one stroke unless other stocks of the same strain exist.

As a general rule an inbred strain must be maintained by continuous full brother × sister matings. Members of different litters may be mated, as long as both parents of the two litters are the same. But it is probably safer to mate only members of the same litter because mistakes are less easy to make. Every precaution must be taken to ensure that the mated pairs are full brothers and sisters. A male may be mated to several of his sisters, but care must then be taken that the offspring mated are full and not half-brothers and sisters.

Ideally an inbred strain is maintained by the single-line system of inbreeding. But in practice this is impossible because not enough pairs can be made up from a single family. Therefore a number of parallel lines must be developed. These sub-lines should, however, not be allowed to run too far in parallel. The least productive should be weeded out and replaced by the expansion of the more productive sub-lines, so that none of the sub-lines in the stock are separated from each other by more than about ten generations.

Now, mistakes do happen, even with the best precautions, and we must consider the consequences. A stock of an inbred strain is a single line. So from what has gone before we see that an outcross with a different strain will undo all the inbreeding and thus destroy the inbred strain. Crosses with other stocks must, therefore, be made impossible. Crosses that are not brother × sister but are within the strain itself will not undo the inbreeding, and therefore do not have serious consequences. If the cross is between two sub-lines, the inbreeding is undone as far back as the point at which the two sub-lines diverged from each other. And if this is no more than about ten generations previously, the consequences are not disastrous. So if an error in mating is detected, no drastic steps need be taken so long as it is absolutely certain that the erroneous mating was between two individuals of the strain. The progeny of the erroneous mating are still inbred animals of that strain, and can be sold as such. But it would be better not to use them for breeding because the breeder should be able to swear con-

scientiously that his stock has been maintained throughout by continuous brother-sister matings.

3. SOME COMMON CAUSES OF INFERTILITY IN LABORATORY ANIMALS

H. M. Bruce

(A) Introduction

A knowledge of normal breeding for any particular species is essential for the recognition of infertility. For the purpose of this note infertility is applied to the breeding colony as a whole and not to the individuals of which it is composed, and hence to relative failure in production, whatever the cause. The practical aim in the breeding of laboratory animals is the raising of young suitable for experimental work. Survival after birth is clearly distinct from capacity to give birth to living young, but because both contribute to the fertility of the colony in the economic sense of the word, the broad interpretation of fertility is justified here.

Many factors essential for fertility are not directly connected with the physiology of reproduction. This note is an attempt to review some of them.

(B) Diet: Faults in Quality

Absolute or relative deficiencies will reduce fertility either directly by specific effects on the reproductive cycle, or indirectly by lowering vitality. Dietary requirement of essential factors is a question of balance rather than of fixed amounts.

Gross deficiencies are unlikely in mixtures of freshly prepared natural foodstuffs because of the wide distribution of nutrients, but there may be unsuspected defects which limit production.

"Alterations in a stock diet for rats which had been in use without modification for years, led to increases of 20% to 30% in litter size, in weaning rate and in the weight of young weaned" (Mendel and Hubbell, 1935).

1. protein

Protein, being generally the most expensive ingredient in a stock diet, must often be kept as low as possible in the interests of economy. Restricted sources of protein involve the risk of amino-acid deficiency;

for example, casein, the main protein in milk, contains very little cystine. "A diet containing 17·7% casein and 3·7% dried yeast was inadequate for lactation in rats, unless it was supplemented with cystine" (Sure, 1941).

2. VITAMIN E

Species differ greatly in their need for vitamin E. On severely deficient diets male rats are rendered permanently sterile but male mice are unaffected. Male rabbits show muscular degeneration but no testicular damage. Female rats, mice and guinea-pigs suffer reproductive disturbances. In acute deficiency, death of the foetus takes place during gestation and few litters are born alive; in mild deficiency, the young fail to survive. Vitamin E deficiency may be cumulative and the effect not manifest for several generations.

3. VITAMIN A

Reproductive disturbances also follow a deficiency of vitamin A. Gestation may be prolonged and parturition difficult. Lactation is poor and weaning rate low. Testicular damage occurs in the male, but is not permanent.

Loss of both vitamin A and vitamin E may develop on storage in diets containing ingredients liable to become rancid or having a high content of unsaturated fatty acids. "When the cod liver oil content of a stock diet for rats and mice was increased from 1% to 2%, the diet became vitamin E deficient" (Bruce, 1950*a*).

4. VITAMIN C

Monkeys and guinea-pigs are the only laboratory animals which must be supplied with vitamin C in the diet. Vitamin C is unstable and is rapidly lost on storage. It is probably the most important single factor responsible for impaired fertility in guinea-pigs. The requirement for pregnancy is more than twice that for maintenance or growth. In mild deficiency, litters are small and there is a high rate of stillbirths; in severe deficiency, litters are aborted or resorbed.

5. VITAMIN B_1 AND MEMBERS OF THE VITAMIN B COMPLEX

Evidence is accumulating that members of this class are concerned specifically at certain stages in the reproductive cycle, and various disturbances in breeding are likely if the diet is deficient. The females may not come into heat regularly in the normal way, the development of the young during pregnancy may be affected and result in congenital deformities at birth, conception rate may be low and foetal mortality high.

Diets theoretically adequate and accepted as satisfactory in practice may yet lack some of these factors. "In the Cornell Rat Colony, U.S.A., a commercial calf meal was in use as a stock diet for rats for 14 years before its limitations were recognized" (Loosli, 1945).

(C) Diet: Faults in Quantity

General malnutrition reduces vitality and impairs fertility.

1. insufficient food

Feeding wet mash diets which become sour before the animals have eaten their fill or infrequent feeding of dry diets in open pots which are easily fouled so that the food is refused by the animals may lead to chronic underfeeding. "Restriction of calorie intake by one-third that of a control group with unrestricted feeding, lowered the pregnancy rate in female mice by nearly 70%" (Ball, Barnes and Visscher, 1947). "When the calorie intake was restricted by one-half, oestrous cycles ceased" (Carr *et al.*, 1949). "Males on restricted calorie intake sired smaller litters than the fully fed controls" (Lee *et al.*, 1951). "In rats, when the energy intake fell below 70% that of a fully fed group, fertility was impaired, and when it fell below 60% the animals were sterile" (Escudero *et al.*, 1948).

2. insufficient water

In the normal animal, food and water intake are closely parallel, restriction of the one immediately resulting in a reduced intake of the other. Where frequent failures in the supply of drinking water occur there is the associated risk of chronic underfeeding. "Rabbits and guinea-pigs given unlimited fresh green food as a source of water and none to drink were unable to consume sufficient greens to meet their need for water. Less food was eaten and growth was slowed. Similar effects were seen with mice fed only on a wet mash" (Bruce and Parkes, 1946; Bruce, 1950*b*).

(D) Disease

The organisms responsible for many of the common diseases of laboratory animals are likely to be present in most colonies. They exist in certain individuals which are carriers of the particular disease and are not affected by it. Under adverse conditions, susceptible individuals become infected and overt disease develops.

Chronic infections of such diseases as mouse arthritis, *Streptobacillus moniliformis*, and mouse typhoid, *Salmonella typhimurium*, from which

infected animals may recover to become re-infected later, have a detrimental effect on colony fertility by reducing the number of productive individuals.

The reasons governing the outbreak of disease in a colony previously healthy are obscure, but it seems likely that the general levels of hygiene, housing and care are among the most important.

External parasitic infections do not affect fertility directly. Widespread parasitic infection reflects poor hygiene either past or present and is likely to be accompanied by debility and poor reproduction.

(E) Environment and Husbandry

Defects in general environment and in husbandry may affect the fertility of a colony.

1. temperature

Animals do not thrive in draughts or if they are subjected to sudden fluctuations in temperature. Small day-to-day variations of a few degrees are probably helpful in maintaining vigour. Overheating is more harmful than cold. "Fertility of mice kept at 90-91°F was impaired by comparison with that of litter mates kept at 70°F. At the higher temperature sexual maturity was delayed, litter size reduced and still-birth rate increased; second generation mice were sterile but their fertility was restored by transfer to the cold; nutritional requirements of both rats and mice were increased (e.g. vitamin B_1 twofold, choline ten-fold); growth was retarded and resistance to infection reduced" (Mills, 1945).

2. light

Shortened hours of daylight with insufficient artificial light probably contribute substantially towards the seasonal depression in fertility common to many laboratory animals. "During the winter months mating was improved in one rat colony by increasing the light from 8 to 13 hours per day" (Alexander and Frazer, 1952). "Decreasing the light from 15 to 9 hours per day reduced breeding to a minimum in a colony of field mice kept under laboratory conditions" (Baker and Ranson, 1932). "Age at sexual maturity in female rats is influenced by light. Females reared in continuous light became sexually mature about a week earlier, and females reared in the dark about three weeks later than those reared under normal conditions of light" (Fiske, 1941).

3. NOISE

Unusual disturbance is liable to have an adverse effect on colony production. "Maternal care was diminished in rats subjected experimentally to noise, but mating was not affected" (Farris and Yeakel, 1944).

4. HANDLING

Reproductive efficiency may be reduced if the animals are handled without due consideration. "Improved lactation resulted from changes in the method of housing and handling in two strains of mice" (Fenton and Cowgill, 1948).

5. OVERCROWDING

In crowded conditions colony production is impaired because some individuals fail to conceive, young animals grow more slowly, and mortality increases.

6. HUSBANDRY

In some species the female comes into heat shortly after parturition. In such species reproduction will be well below the maximum of which the animals are capable if the pregnant females are isolated from the males. "In a guinea-pig colony production was reduced by about 40%" (Rowlands, 1949) "and in a mouse colony it was halved" (Bruce, 1947) "when there was no opportunity for conception to take place at *post-partum* oestrus."

REFERENCES

Alexander, P. D. and Frazer, J. F. D. (1952). *J. Physiol.* **116**, 50.
Baker, J. R. and Ranson, R. M. (1932). *Proc. Roy. Soc.*, B. **110**, 313.
Ball, Z. B., Barnes, R. H. and Visscher, M.B. (1947). *Amer. J. Physiol.* **150**, 511.
Bruce, H. M. (1947). *J. Hyg., Camb.* **45**, 420.
Bruce, H. M. (1950*a*). *J. Hyg., Camb.* **48**, 171.
Bruce, H. M. (1950*b*). *J. Anim. Tech. Ass.* **1** (3).
Bruce, H. M. and Parkes, A. S. (1946). *J. Hyg., Camb.* 44, 502.
Carr, C. J., King, J. T. and Visscher, M. B. (1949). *Fed. Proc.* **8**, 22.
Escudero, P., Herriaz, M. L. and Musmanno, E. (1948). *Rev. Assoc. argent. Dietologia* **6**, 195.
Farris, E. J. and Yeakel, E. H. (1944). *Anat. Rec.* **89**, 325.
Fenton, P. F. and Cowgill, G. R. (1948). *Proc. Soc. exp. Biol., N.Y.* **68**, 58.
Fiske, V. M. (1941). *Endocrinol.* **29**, 187.
Lee, Y. C. P., King, J. T. and Visscher, M. B. (1951). *Amer. J. Physiol.* **167**, 375.
Loosli, J. K. (1945). *Ann. N.Y. Acad. Sci.* **46**, 45.
Mendel, L. B. and Hubbell, R. B. (1935). *J. Nutr.* **10**, 557.
Mills, C. A. (1945). *Ann. N.Y. Acad. Sci.* **46**, 97.
Rowlands, I. W. (1949). *J. Hyg., Camb.* **47**, 282.
Sure, B. (1941). *J. Nutr.* **22**, 491.

8

Hygiene

1. STERILIZATION AND DISINFECTION

A. A. Tuffery

(A) Definitions

1. Sterilization is the act or process of sterilizing or freeing from all living organisms.

2. Disinfection is the killing of organisms by the application of chemical or physical agents to contaminated objects.

Sterilization in the animal house is necessary in order to destroy infected material derived from infected animals. Animals may be infected during the course of experiments or, accidentally, during naturally occurring (intercurrent) infections. Pathogenic (i.e. disease producing) organisms occur among the viruses, bacteria, fungi and protozoa, and so long as the sterilizing conditions used are adequate to kill the most resistant members of these groups one can be sure of achieving a satisfactory hygienic routine. Other groups or organisms (nematodes, mites, etc.) will all be killed under these conditions. Spore-forming bacteria, e.g. *Bacillus anthracis* (causative organism of anthrax), are probably the most resistant organisms one is likely to require to kill—if the conditions used are adjusted so as to kill this type of organism then all other organisms will also be killed. Various methods used to achieve sterilization will be described in turn, but one or two general factors must be kept in mind in order that sterilization can be carried out efficiently.

First of all the sterilizing agent used must be able to get to the infected material, e.g. heat must be applied for a sufficient length of time for it to first penetrate and then sterilize the layers of bedding material used in cages. Secondly the conditions used must be bactericidal and not merely bacteriostatic, that is, they must kill bacteria and not just inhibit or prevent them temporarily from multiplying. This last state of affairs is particularly liable to occur when chemical disinfectants are used. The disinfectant may become very dilute or be partially neutralized by soil from previously treated cages. The lethal action of a disinfectant is due in the main to its capacity to react with the protein and in particular the enzymes of micro-organisms. Any

agent therefore which will coagulate, precipitate or otherwise denature proteins will act as a general disinfectant. Among such agents are heat, chemicals and irradiation.

(B) Methods of Sterilization

1. heat

Heat is probably the most important agent used for the destruction of micro-organisms. In general, bacteria are more susceptible to moist heat than to dry heat. Sterilization with moist heat depends largely upon coagulation of the cell proteins, whilst sterilization with dry heat depends more on the oxidation of cell components for its effect. In general, among bacteria which are parasites of mammals, non-sporing forms in a moist state cannot stand temperatures above 45°C for any length of time. Some species however (those of the *Bacillus* group) develop highly resistant spore stages which are capable of surviving this and considerably higher temperatures for a comparatively long time.

i. Moist heat. The application of moist heat by the means of an autoclave or by what is usually referred to as free-steaming are probably the most commonly used sterilizing techniques in the animal house.

Free-steaming is perhaps the simplest method of sterilizing cages. Cages and any other equipment which will withstand this treatment can be either sprayed with jets of steam in a tunnel washer or placed in a dairy type sterilizer. This consists of a large galvanized iron chest into which steam is passed or in which steam is generated by boiling water in the lower part of the apparatus. Theoretically one is able to achieve temperatures higher than 100°C, in practice, however, temperatures are unlikely to reach very much above 85°C, but experiments have shown that if steaming is carried out for sufficient length of time (under most conditions 1 to 1½ hours) this treatment will kill *Salmonella typhimurium* and even tubercle bacilli in shallow layers of litter in the bottom of cages (Committee Report, 1957).

In free-steaming processes the steam is applied to the cages under more or less normal atmospheric pressure. In the autoclave steam is applied under an increased pressure, here the object is to supply moist heat to the surfaces to be disinfected at a temperature greater than 100°C.

The temperature at which water boils, and therefore steam is formed, depends upon the pressure of the air or steam above the surface of the boiling water. If one can increase this pressure, the temperature of the

boiling water (and therefore the steam) can be increased as shown in the table. The autoclave is simply a device for holding articles to be sterilized in an atmosphere of steam under pressure. The pressure generally employed is 15 lb per square inch, at which pressure saturated steam attains a temperature of 120°C. Thirty minutes' exposure to this temperature is usually sufficient to kill all forms of micro-organisms including bacterial spores.

Temperatures of saturated steam under pressure

Lb pressure on Autoclave	*Degrees F.*	*Degrees C.*
5	227·1	108·4
10	239·4	115·2
15	249·8	121·0
20	258·0	126·0

Autoclaving is undoubtedly the safest and most efficient way of sterilizing almost any kind of apparatus and is most essential where equipment has held animals infected with organisms which might be dangerous to man.

2. CHEMICAL

For disinfection to occur there must be a chemical reaction between the disinfectant and the micro-organism. The disinfectant has to actually touch the organism; if the organism is embedded in cage dirt, no contact is made and no disinfection results. All soil should be removed from cages before attempting to use a disinfectant for sterilization. Organic matter may either cover the organism so that the disinfectant cannot reach it, it may neutralize the disinfecting action by combining with the disinfectant chemically or it may absorb the disinfectant and thus reduce the amount of active disinfectant. Disinfectants work best when they and the surfaces to be disinfected are warm. The acidity or alkalinity of the disinfectant or the surface to be disinfected should be considered in the selection of disinfectant; for example a hypochlorite solution is most active in slightly acid conditions while the quaternary ammonium compounds are generally most efficient under alkaline conditions.

Generally, single disinfectants work best. For example, soap may act as a "wetting agent" and enable one to clean the surface more thoroughly but it will at the same time react with and neutralize a good many disinfectants.

It is also possible to sterilize material by means of gases such as formaldehyde vapour or ethylene oxide, but these techniques are unlikely to be used in the normal animal house.

i. Phenol and cresol compounds. Phenol is not used much these days as a disinfectant. Cresol compounds and cresol-containing solutions are more usually used. The optimum concentration is about 5% for most of these. This type of disinfectant is cheap and readily obtained and destroys nearly all bacteria and other organisms except those which form spores, functioning chiefly as a protein denaturant.

ii. Hypochlorites and Halogens. Hypochlorites and the halogens (chlorine and iodine) are in general very good disinfectants. Hypochlorites particularly are cheap and readily obtained; they are however corrosive and are not always recommended for use on metal equipment in the animal house for this reason. They are also very readily neutralized by organic matter so that they are used more efficiently on items that have already been cleaned in the usual mechanical sense.

iii. Surface active compounds. Surface active compounds (i.e. compounds which in water solutions lower the surface tension of water and thus increase the wetting capacity of the solution) have achieved great prominence as disinfectants within the last few years. They are highly bactericidal at comparatively high dilutions, are of low toxicity and these days are readily available. There are several different groups of chemicals which fall within this category. Natural and many synthetic soaps are classified as anionic surface active compounds, but these are in general poor bactericides. Quarternary ammonium compounds such as cetrimide and benzalkonium chloride are on the other hand classed as cationic substances. A third group which has recently come into prominence are the ampholytic agents such as *Tego* and a number of other chemicals which are not generally available to the public at present. There are subtle physical-chemical differences which differentiate these groups of compounds (quarternary ammoniums and ampholytes), but so far as the animal house is concerned they have much the same properties. They are relatively non-toxic and capable of leaving residues on treated surfaces that both retain some bactericidal properties and enable subsequent cleaning operations to be carried out more easily. They are also fairly efficient detergents and help in this way to clean the surfaces mechanically. This is an extremely useful property from the point of view of cleaning animal-house walls, shelves, cages, tables and similar apparatus. In general they are used in concentrations of about 1% and their activity is increased if they are used

hot. Perkins and Short (1957) have described the use of one of these compounds for general animal-room cleaning purposes. The technique

TABLE I. Common British Disinfectants

Class or Type	*Industrial Name (U.K.)*	*Manufactured by*	*Effective against*	*Notes*
Chlorine Iodine	—	—	Most bacteria	Usually used as the hypochlorite but not in the animal house
Hypochlorites	Chloros	I.C.I.	Most bacteria and viruses	Cheap; effective; somewhat corrosive and not usually recommended for repeated use on metal items; rather readily neutralized by soil, faeces, etc.
	Sod. hypochlorite (10% w/w Chlorine)	Hopkin and Williams	Most bacteria and viruses	As above
Cresol	Lysol B.P.	Jeyes	Most bacteria including *Myco. tuberculosis*	50% Cresol in soap solution. Good general disinfectant
Cresol and Chlorxylenol	Jeypine and other brands	Jeyes	Nor spores or *Ps. pyocyaneus*	General disinfectant, must be used in sufficient concentration. Compatible with soap
Quarternary ammonium compounds	Monidet 32G	Shell	Most bacteria	A very useful bactericidal detergent for general use. Not to be mixed with other materials, e.g. soap
	Cetavlon	I.C.I.	Most bacteria and viruses	As above; low toxicity; useful for water bottles
	Cetrimide BP	Glovers	As above	As above
Ampholytes	Tego MHG	Hough, Hoseason	Most bacteria	As above

described is to be recommended although probably other chemicals might be found which are just as efficient as *Tego*.

There is a wide variety of chemical disinfectants on the market, some of which are listed in Table I. In general, one should use these according to the makers' instructions, bearing in mind the points listed above.

3. IRRADIATION

Finally, it may be noted that under certain conditions various forms of radiation can be used for sterilization, but in general these methods are inapplicable to animal house conditions.

REFERENCES

Committee Report (1957). *Mon. Bull. Minist. Hlth Lab. Serv.* **16**, 64-71.
Perkins, F. T. and Short, D. J. (1957). *J. Anim. Tech. Ass.* **8**, 10.

2. BEDDING FOR LABORATORY ANIMALS

G. Porter

(A) TYPES OF BEDDING

1. SAWDUST

Supplies should be obtained from a modern mill where the sacks are filled direct from the sawbench through the sawdust extractor chute. Sawdust obtained in this manner is unlikely to be contaminated by cats, dogs and wild rodents. It should be clean, dry and absorbent. Wet, dirty sawdust from a mill dump should never be used.

Teak, mahogany and other hard resinous woods contain phenolic substances which may be harmful to animals. Soft or whitewood sawdust is more easily obtained and can generally be used with safety.

2. PEAT MOSS LITTER

Peat moss litter has a high acid content which delays decomposition of faecal matter and the release of ammonia. This reduces smell in animal houses, and when cages are bedded down with peat moss litter they require cleaning less frequently. It is obtainable from fodder merchants in half-hundredweight sacks or bales weighing a hundredweight. The sacks contain granulated peat and are easier to handle than the compressed bales, which, however, are less pervious to infestation. Many users regard peat moss as dirty and dusty, but it is no more so than sawdust, the only difference being the colour.

3. STRAW

Clean oat-straw as used for feeding farm animals is a better bedding material than the cheaper but far coarser wheat and barley straws. Oat-straw is not so hard as other straws and therefore is less dangerous to the animals' eyes, but even so if used in cut form it must be cut to a degree of fineness. Discretion must be exercised in the amount of straw used. Often young guinea-pigs get lost in it and are crushed by their parents, especially in the larger runs.

4. OAT CHAFF AND CAVINGS (OAT HUSKS)

This is quite a popular bedding, used on top of sawdust or peat moss for rabbits and guinea-pigs. The danger of contamination by vermin and cats is ever present. The method of storage on farms usually takes the form of a thatched dump, the breeding ground of vermin and the hunting ground of cats. Very few users have facilities for sterilizing these commodities, but the use of them without sterilization may introduce infection.

5. MEADOW HAY

Generally used as fodder, it does, however, make a good bed for breeding rabbits—especially during cold weather—as the soft hay makes a close and warm nest for the young.

B) STORING

All bedding materials must be stored in a dry well-ventilated ermin-proof building. Sawdust and peat moss may be kept in large bins of the coal-bunker type. Woodwool, hay and straw should be placed on racks well off the floor to avoid contamination.

(C) BEDDING OF ANIMALS IN TRANSIT

To ensure their arrival in good condition animals in transit must be treated with special care. All travelling boxes should contain a layer of at least 2 in. of softwood sawdust with a liberal covering of woodwool or soft hay, depending on the species of animal. Points to bear in mind are (*a*) the necessity to counteract shock caused by handling of the boxes, (*b*) the need of privacy for the animals from interested spectators, (*c*) the importance of keeping the animals clean and dry. For this purpose absorbent material is necessary.

(D) DISPOSAL OF BEDDING

Uninfected bedding is not generally regarded as a source of danger or infection, but it should be burned along with all other materials

from cages. If placed in pits or similar dumps it provides a breeding ground for various types of flies.

Sterilization of infected bedding *along with the cage* must be regarded as a necessary routine. After sterilization the bedding should be removed immediately and burned, the cage may again be sterilized and finally sent for cleaning. Where this is not possible, removal of infected bedding *must* take place in the infected room and the bedding placed in a bin with a lid, then taken direct to the incinerator. The bin and all utensils used in cleaning must also be sterilized chemically or otherwise.

3. FEEDING, BEDDING AND HYGIENE

Gwendoline G. Weeks

(A) Introduction

This article, dealing with the feeding, bedding and hygiene of mice, guinea-pigs and rabbits, does not attempt to cover every aspect of the subject. I have tried to give a summary of the methods and practices adopted by animal-house workers, which they have found from their experience to be expedient in rearing good healthy stock.

(B) Feeding

The need for a diet complete in the nutritional requirements for a particular species cannot be overstressed. Deficiency of an essential vitamin or nutrient may lower the general health of a colony and the resistance of the stock to disease.

There are generally three methods of giving feed to laboratory animals. First as a mash or paste, second as a dry powder, and third as a compound pellet. The last is the method recommended. Souring of the food, which may occur when a mash is fed, is prevented; scattering and waste are greatly reduced; the pellets can be fed in hoppers which prevent the food from becoming contaminated by faeces and urine. This last point is most important in controlling the incidence of intestinal disease in a colony. The labour saved in feeding and preparing diets is also a point in favour of a compressed cubed diet.

If it is possible, diet should be prepared in a room set aside from the animal rooms. All feed, especially green-stuff, should be obtained from a reliable dealer who has not allowed it to come in contact with vermin. The adequate storage of diet is a point often overlooked.

Protection should be provided from flies, cats, dogs, rats, mice, damp, heat, light and excessive oxygen, which may effect the vitamin content of a diet. Covered metal bins have so far proved the most satisfactory for storing diets.

The regular feeding and watering of animals is often overlooked. The ideal is to feed the animals at the same time every day.

A fresh constant supply of water is essential for the well-being of stock. Kennaway (1943) and Bruce (1950) provided evidence that the moist food system adopted as a substitute instead of water is unsound. Even when a wet mash or succulent is given, animals will still drink water. Open water pots placed in the cage are liable to contamination by faeces and urine. Individual fountains or water bottles are more hygienic than a common supply system, since, if infection occurs, it is more difficult to control using this latter method of watering. The water and nutritional requirements are higher during the reproductive phase of an animal's life. This is especially so during the lactation period; if feeding is not *ad lib.*, extra diet should be given during these stress periods.

Any change of diet, should this be necessary, must be introduced gradually. Violent fluctuations in the nature of a diet may have serious effects on a colony. Caution should be taken to avoid feeding frozen, poisonous, soiled, decayed or contaminated food, diseased or mouldy material. Food should not be cooked in galvanized buckets, in view of the possibility of zinc poisoning (Ormad, 1943).

1. GUINEA-PIGS

A glance at the *Catalogue of Uniform Strains*, published by the Laboratory Animals Centre, will give some idea of the variety of diets that are fed to laboratory animals. Obviously if you are using a feed that suits your strain and is giving good results, it would be rash to introduce drastic changes. The diet fed, however, must contain the known nutritional requirements for that species.

Unlike other small laboratory animals, guinea-pigs are unable to synthesize vitamin C; it is most essential that an adequate amount of this vitamin should be supplied daily, otherwise scurvy will develop. Owing to it being readily oxidized, vitamin C is not generally incorporated in a pellet diet. It is present in fresh kale and cabbage, but it is not adequately supplied by root vegetables in the winter. The vitamin C requirements are related to body weight. A guinea-pig weighing 200g needs 2·5mg of ascorbic acid daily; an 800g pig

requires 10 mg daily. A safe allowance for an adult is 13-15 mg per day.[1] The requirement of pregnant sows is approximately twice this amount while 2 oz per head per day of cabbage will satisfy the vitamin C requirements of a non-pregnant guinea-pig. The cabbage should be washed before feeding. Fresh-cut young grass provides a source of vitamin C, but care should be taken to see that it does not become heated if left overnight. Of the other vitamins, A has been shown to be necessary; D is required if the calcium-phosphorus ratio is different from 1; 1·5 mg. of vitamin E should be supplied daily.

Alfalfa or lucerne hay is high in calcium and vitamin A. An adequate supply of vitamins A and D and calcium aid bone growth and help to build up a resistance to colds and pneumonia (Ibsen, 1927).

Guinea-pigs do not readily eat dry, powdery diets. It is generally more advisable to feed pellets or a mash. Diet 18 (Bruce and Parkes, 1947), and Diet S.G.1 (Short and Gammage, 1959) are standard pelleted diets for guinea-pigs.

Formula Diet 18

	Percentage	*Theoretical Composition*	*Percentage*
Bran	15	Protein	16·5
Dried grass meal	30	Fat	4·6
Ground nut cake	15	Carbohydrate	33·7
Linseed cake	10	Fibre	6·7
Dried meat and bone meal	8		
Barley meal	20		
Sodium chloride	1		
Calcium carbonate	1		

Formula Diet S.G.1

	Percentage	*Theoretical Composition*	*Percentage*
Best White fishmeal 67% Protein	10	Protein	14·692
Grassmeal 18% Protein	20	Fat	3·4
Bran	40	Carbohydrate	36·618
Sussex ground oats	12	Fibre	4·508
Middlings	18	Ash	7·318
		Lime	1·578
		Phosphoric Acid	2·537
		Potash	1·578
		Chlorine	·430

[1] See footnote, page 107.

This diet requires a daily supplement of vitamin C. A supply of good-quality meadow hay should be available all the time to provide roughage in the diet. The nutritive value of the hay will be partly dependent on the stage of growth at which it was cut and losses that have occurred during drying and storage. Hay should be greenish to light yellow-brown in colour, with a pleasant smell. Overheated hay with a mouldy appearance should be discarded. All hays provide valuable minerals; calcium, potassium, sodium, magnesium, phosphorus, chloride and sulphur, which are required for healthy growth.

Even if succulents are fed, guinea-pigs still require a constant supply of fresh water. The water requirement is related to body weight. Guinea-pigs are constant feeders, and do not eat a lot at a time, therefore it is best to keep food and water before them continuously.

2. RABBITS

In the wild state, rabbits are herbivores, feeding on legumes, grasses and suchlike food. For successful production, however, rabbits kept for breeding require in addition to green-stuff and hay a concentrated supplement containing protein. If fed without supplement to lactating and growing stock green foods, owing to their low energy content in relation to bulk, cause the animals to become pot bellied and thin.

The Morant system has been tried at some breeding stations. Only the healthiest and strongest rabbits are kept. Movable hutches with wire-netting runs are used. The rabbits can crop the grass through the wire netting. The folds are moved each day, giving rabbits access to fresh green. During the winter, if snow makes the natural diet inaccessible, a mash and cabbage is fed.

The exact nutritional requirements are as yet poorly understood. Templeton (1939) says that a diet with 12-15% protein is adequate for resting stock; 16-20% protein in the diet is required for reproducing does.

It was once thought that the great length of the small intestine in a rabbit was a provision for dealing with bulky foodstuffs and enabling the animal to utilize fibre. It is now known that fibre is poorly utilized in these animals in comparison with ruminants.

Protein metabolism is influenced by coprophagy. This coprophagy probably supplies the rabbit with B vitamins formed by microbiological synthesis in the caecum and large intestine. Unlike guinea-pigs, rabbits are able to synthesise vitamin C. Vitamins A and E are required in the diet. The exact requirements of the other vitamins have not been worked out yet.

If cod liver oil is added to a diet, care should be taken to see that it

does not cause oxidative destruction of vitamin E in the diet. Excess cod liver oil causes injury which appears to be identical with nutritional muscular dystrophy. Cod liver oil can be fed on cabbage leaves, or as a supplement with bran before the main food.

Green foods vary in the feeding value, but of these kale and cabbage are the least variable. Scythed grass decreases in nutritional value as it becomes old and stemmy; as the fibre content rises the digestibility declines. Green foods contain 80-85% or more moisture; if they are fed too liberally they cause excessive urination, and the cages smell. Sugar beet should be wilted at least a week before feeding; it may be harmful if fed extensively while fresh, owing to the high oxalic acid content. Yellow shoots which may grow on the roots during storage should be removed before feeding. Raw potatoes are injurious if fed constantly, especially to young stock and pregnant does.

Hay should not be used indiscriminately as a substitute for fresh greens. Excess may result in paralysis and breeding failure. Legume hays, clover and alfalfa (lucerne) can be fed in addition to succulents.

Grain mixtures are suitable maintenance supplements, but for breeding stock and weaners, additional protein can be supplemented, for example linseed and soya bean supplement.

The absence of concentrates during pregnancy results in a lower birth weight of the young. If natural grains and hay are fed, salt should be provided in blocks on the side of the cage.

A constant supply of fresh water is a dietary essential. The daily requirement is about one-seventh of the body weight. Like most rodents, rabbits are normally more active during the night; they eat more during the night than during the day. Where grain and hay are both fed, feed grain first thing in the morning, and hay and pelleted food in late afternoon.

Diet 18 and S.G.1 as described for guinea-pigs can also be fed to rabbits as a complete diet for growing and breeding.

If they are overfed, the animals become fat and sluggish. This also suggests uneconomical management. An adult should be impatient for food at feeding time. Young growing stock can have grain and hay before them all the time.

Coccidiosis infestation was lowered in the U.S.A. in domestic rabbits when the dietary regime was changed from green and root feeding to prepared stock diets. Coccidia may contaminate green foods. During the preparation of pelleted diets, the cubes are exposed to high enough temperatures to inactivate the cysts.

Rabbits need fibre in the diet, but not to excess, because physically they are unable to eat sufficient bulky and indigestible material in twenty-four hours to satisfy their needs; also the energy used in mastication and digestion of the food would be greater than the nutrient obtained. Ten per cent. fibre is sufficient in a ration.

3. MICE

Unlike rabbits and guinea-pigs, mice do not require excess roughage in their diet. Therefore hay is not a dietary essential. Since they are able to synthesize vitamin C, they do not require succulents. The most hygienic and economical way of feeding mice is to give a pelleted diet. This can be fed in a food hopper, thus preventing the mice from spoiling the diet with faeces and urine. There are several compound pelleted diets which will supply adequate nourishment for the breeding and growth of mice. The constituents and theoretical compositions are listed below.

Formula Diet 41 (Bruce, 1950)

	Percentage	*Theoretical composition*	*Percentage*
Whole meal flour	46	Protein	13·7
Sussex ground oats	40	Fat	3·5
Fish meal	8	Soluble carbohydrate	49·0
Dried yeast	1	Fibre	1·5
Dried skimmed milk	3	Ash	not given
Cod liver oil	1	Calcium-phosphorus ratio	not given
Sodium chloride	1	Moisture	not given

"The destructive effects of cod liver oil on Vitamin E is well known and as a precaution since June 1956 the cod liver oil in Diet 41 has been replaced by stabilized vitamin supplements (Alpha Beta 8) and a small amount (about 3%) of molasses used to bind the cubes. This modified diet is known as Diet 41B" (Bruce and Parkes, 1956).

Formula Diet 86 (Howie, 1951)

	Percentage	*Theoretical composition*	*Percentage*
Wheat, whole ground	50	Moisture	14·3
Barley, whole ground	25	Soluble carbohydrate	53·4
White fish meal	7	Protein	20·0
Meat and bone meal	6	Fat	3·8
Dried brewer's yeast	5	Fibre	3·3
Dried grass meal	5		
NaCl	1		
Cod liver oil	1	Ash (Calcium 0·7; Phosphorus 0·8)	5·2

Formula Thomson Cubed Stock Diet (Thomson, 1936)

	Percentage	*Theoretical composition*	*Percentage*
Wheat offal (fine middlings No. 2)	19·2	Protein	14·9
Ground wheat	19·2	Fat	not given
Sussex ground oats	19·2	Soluble carbohydrate (as starch value)	65·9
Ground barley	9·5	Fibre	not given
Ground maize	9·5	Ash	not given
Meat and bone meal (45% protein)	9·5	Calcium-phosphorus ratio	1:1·19
Dried skimmed milk	7·0	Moisture	not given
White fish meal (60% protein)	4·7		
Dried yeast (46% protein)	1·2		
Sodium chloride	0·5		
Cod liver oil	0·5		

It is known that a strain difference does occur in the reaction to diets. Some may do very well on a particular diet, but others may produce litters or not rear the litters to weaning. If a high mortality rate at the weaning period is experienced, the adequacy of the diet should be questioned. The vitamin requirements of mice have not yet been fully investigated. Vitamins A and D are needed, but the amounts required have not yet been determined. All the components of the vitamin B complex are thought to be necessary; these will be provided if the diet contains a good percentage of cereals, e.g. whole wheat and oats. Brewer's yeast will also supply the B vitamins. It is thought that the animal is able to synthesize its own vitamin C requirements. From information supplied by the *Catalogue of Uniform Strains*, the majority of laboratories breeding mice use one of the standard pelleted foods, with excellent results. Mice require a good supply of fresh water, about a quarter of their body-weight daily is needed. This should be supplied in water bottles, care being taken to see that young weaners can reach the water spout.

(C) Bedding

Clean and uncontaminated bedding is as important in the care and maintenance of a breeding colony as proper housing, cages and diet. All bedding material should be obtained from a reliable source, where strict measures are taken against exposure to wild rodents, cats and dogs. Bedding bought from an unreliable source may introduce

infection, fleas and lice into an otherwise healthy stock. If it is possible, all materials should be sterilized before use and precautionary measures taken against contamination.

The type of cage used will determine whether or not the animals are in contact with the bedding. If cages with solid floors are used, the animals will be in contact with the bedding, and even greater care must be taken to see that it is uncontaminated. Animals housed in cages with mesh grid floors are not in contact with the bedding, and the need for its sterilization is not so great on condition that it has been obtained from a reliable source.

Bedding can be classed under two headings, namely bedding used as an absorbent and bedding used for nest making.

i. Absorbent types of bedding. Sawdust, peat moss and wood shavings are the most common types of absorbent beddings. Hay and straw are less commonly used.

Soft or whitewood sawdust is preferable; it is dry and absorbent and can be used in all types of cages and for all animals. Sawdust from a mill dump should never be used as it may have been contaminated by cats and vermin; also wet sawdust should be avoided.

The advantage of peat moss litter is that it has a high acid content which delays decomposition of the faecal matter and the release of ammonia and therefore reduces smell. Short (1951) found that the interval time for cleaning was extended to twice that of other beddings when peat moss was used. It can be used as a bedding for mice, guinea-pigs and rabbits. Wheat chaff can be mixed with peat moss. Peat as bedding will not stain the fur of rodents if the pens are cleaned at least every 10 days to 2 weeks.

For mice, Herrlein (1940) uses white pinewood shavings, baled at the mill, and agricultural peat moss for rabbits and guinea-pigs. Wood shavings can also be used for rabbits.

ii. Bedding used for nest making. Some breeders consider it more desirable to keep animals on litter all the time rather than to expose them continuously to wire grids or pens. The disadvantage is that cages cannot be cleaned so easily and the bedding will be fouled by urine and faeces, which will attract flies. Some form of bedding should be given to pregnant females for nest building.

Woodwool, straw and hay are commonly used as bedding for nest building. No. 1 woodwool is good for species of rodents. It can be supplied by any reliable firm dealing with fruit-packing materials. It is packed in bales, which are impervious to attacks by vermin, beetles,

etc. The baling process is carried out when the wood is cut, so no contamination can have taken place. A blow-lamp can be used on the outside of a tightly packed bale, in case contamination has occurred at the docks or railway stations. This form of nest bedding is to be recommended where facilities for sterilization are lacking.

Hard straws should not be used as they may damage the eyes of the animals. Oat straw is good for guinea-pigs and rabbits. Meadow hay can be used for breeding rabbits, but care must be taken to see that it is not eaten to excess. Hay can also be used for bedding mice. Oat husks are not recommended for rabbits and guinea-pigs because of the likelihood of contamination by vermin and cats.

It cannot be overemphasized that if it is possible all bedding, especially that which comes into direct contact with the animal, should be sterilized before use. It should be bought from a reliable source and stored in a vermin-proof room. Surplus bedding must not be returned from the animal room to the main store. Dirty bedding should be burnt with the rest of the litter. Infected bedding should be sterilized with the cage, then the bedding should be removed to covered bins in the infected room, and the bins taken and emptied into the incinerator. The bin and cages should be sterilized or treated with a blow lamp.

(D) Hygiene

The Oxford Dictionary defines hygiene as "that knowledge or practice which relates to the maintenance of health; a system of principles or rules for promoting health."

Hygiene in the animal house, therefore, is the practice of maintaining a healthy colony. The rules, if they can be called that, really amount to a routine procedure, ensuring as near as possible a constant external environment for the general well-being of the stock. Elaborate equipment for sterilizing and disinfection is not essential for rearing good healthy animals; the regular use of soap and water and the development of regular routine sanitary habits are most important.

It is not generally possible to rear animals free from bacteria or virus pathogens. Infections may be revealed by sudden changes in diet, violent fluctuations of temperature or damp bedding, or any abnormal conditions of environment. The aim of the breeder should be to maintain a colony in as near a constant external environment as possible.

Much could be written on this subject, but I am only going to summarize what I think are the main essentials.

1. The development of a routine procedure by the animal house worker is important. Scrupulous personal cleanliness is essential. If more than one species of rodent is reared, it is advisable to finish cleaning and feeding one group before going on to the next. If an overall can be kept for each group it will help to reduce the risk of carrying disease from one group of animals which may act as carriers to others in which it may be fatal.

2. The feeding of pelleted food in wire food-hoppers prevents fouling by faeces and urine. With proper feeding, animals are resistant to most diseases. The provision of water bottles, as opposed to open dishes, ensures that a supply of fresh clean uncontaminated water is available to the animal. Fouled water may produce diarrhoea. In one animal house the incidence of pseudotuberculosis was reduced by the prevention of contaminated food and an improvement in the general cleaning and disinfection of the feeding utensils.

3. The provision of adequate storage space for food and bedding in vermin-proof bins reduces the likelihood of disease. If possible, a separate room should be set aside for the preparation of diets.

4. Precautions should be taken to see that all animal houses are proof against wild rodents. Wild mice can spread salmonella and pasteurella infections to guinea-pigs. Animals should be housed in dry, draught-free rooms, without violent fluctuations in temperature. Such fluctuations may cause respiratory disorders, especially in mice and guinea-pigs. Mice breed best in a temperature that does not fluctuate more than a degree or two from 70°F. Guinea-pigs prefer a temperature around 65°F. Adult guinea-pigs will survive the winter in unheated houses, but the breeding performance may be impaired. Rabbits are more hardy animals, and they can be kept in outdoor hutches, but the important point is to endeavour to keep them in a constant external environment. Wet, warm cages provide very favourable conditions for the development of coccidiosis in rabbits. If possible, animals should be kept clear of faecal droppings, so that the likelihood of cross-infection is reduced.

5. Prevention is always better than cure in an animal house. Cages, adequate in size for normal exercise and which can be kept in sanitary conditions with a minimum of time and labour, are the ideal, but cleanliness and general hygiene can be practised in breeding establishments lacking up-to-date equipment. Independent units are more easy to clean out and it is easier to control infection should it arise. Regular cleaning of cages, food and drinking receptacles is important. This

operation should be performed in a separate room. When cage cleaning is neglected, damp conditions created by the accumulation of urine and faeces help to provide the spread of parasites and bacteria. Soap, hot water and a chemical disinfectant should be used when steam sterilization is not practicable. The risk of ectoparasites is reduced when cages are sterilized routinely. Sterilization is not to be regarded as a substitute for cleaning, but as a complement to it. Lysol and cresol, used in 3-5% solutions, are examples of chemical disinfectants. The disinfectant can be prepared in a tank, and the cages, etc., can be immersed in it. Most chemical agents are poisonous and care should be taken to wash the disinfected article in running water after removal from the tank. A blow-lamp is an efficient sterilizer, if the flame will not damage the article being sterilized.

6. The accumulation of soiled bedding and stale food attracts flies and other disease-carrying insects. All waste should be placed in covered bins and removed regularly to an incinerator and burnt. Dead animals should be incinerated. Screening of doors and windows helps to prevent the entry of flies, but such precautions are often costly and the results do not warrant the expenditure. Any of the tested fly repellants can be used, providing they are not toxic to the animal. DDT is not recommended because of its toxic effects. Aerosols, which give off a fine spray of insecticide, or electric fumigators which fit into the electric-light socket, are also efficient for keeping away flies.

Gammexane is efficient for the removal of flies and lice. The soluble form can be used to spray floors and walls. Small amounts of the powder can be rubbed into the fur of infected animals, but it is toxic if taken internally. It is safer to use 0·5% pyrethrum as a dusting powder.

7. Early recognition and elimination of diseased animals helps in the control of disease. Post mortems should be conducted on all dead animals; knowing the cause of death aids in preventing the spread of disease, since definite steps can be taken to eradicate it. The free post mortem offered to accredited breeders should be used at the first suspicion of infection.

8. Any animals introduced from outside into a colony should be isolated from the rest of the stock for at least two months and put under observation. If possible, when animals are brought in, space should be

set aside for a quarantine room. The introduction of animals into a closed colony always carries with it the risk of cross-infection.

Many of the points described above may seem very obvious, but it is often the obvious that gets neglected. It is surely worth while considering seriously anything that helps to promote the general well-being and care of breeding animals and will ultimately improve the efficiency of the stock.

REFERENCES

Bruce, H. M. (1950-51). *J. Anim. Tech. Ass.* **1**, 3, pp. 2-8.
Bruce, H. M. (1950). *J. Hyg., Camb.* **48**, 171.
Bruce, H. M. and Parkes, A. S. (1947). *J. Hyg., Camb.* **45**, 70.
Bruce, H. M. and Parkes, A. S. (1956). *J. Anim. Tech. Ass.* **7**, 54.
Davies, C. J. "Rabbits for fur and flesh." Published by *Country Life.*
Ferguson, W. S. (1952). Technical Note No. 3. Laboratory Animals Bureau. "Hays and straws for laboratory animals."
Herrlein, H. G. (1940). "A practical guide on the care and uses of small animals for Medical Research." (New City, N.Y., 2. Rockland Farms.)
Howie, J. W. (1951). *J. Anim. Tech. Ass.* **2**, 7.
Ibsen, H. L. (1927). *Science N.S.* **66**, 509-510.
Kelly, L. G. (1951-52). *J. Anim. Tech. Ass.* **2**, 1, pp. 5-7.
Kennaway, E. L. (1943). *Brit. med. J.* **1**, 760.
Ormad, A. N. (1943). *Vet. Rec.* **55**, 366.
Short, D. J. (1951-52). *J. Anim. Tech. Ass.* **2**, 3, pp. 3-6.
Short D. J. and Gammage, L. (1959). *J. Anim. Tech. Ass.* **9**, 4.
Templeton, G. S. (1939). "Feed Requirements of rabbits." Proc. 7th World Poultry Congress, Cleveland, Ohio. pp. 468-471.

4. THE HYGIENE OF LABORATORY ANIMALS

P. L. Shanks

One dictionary definition of hygiene is that it is "the science which treats of the preservation of health". Good health so far as laboratory animals are concerned is of paramount importance, for not only can an outbreak of an infectious disease decimate a breeding stock or a group of animals under experiment, but minor illnesses can so upset an experiment as to render it valueless. To get the best results both for the breeder of the laboratory animals and for the scientists who use them it is necessary for the animals to be healthy and kept under conditions which will maintain that good health.

Several factors combine to provide satisfactory hygiene for animals. First of all they must be housed in suitable buildings and suitable cages; secondly they should be given adequate food; and thirdly they

should be kept free from disease. It is in connection with the latter that this article deals.

Disease can be caused by a number of different agents. The familiar assumption is that disease is caused by germs, e.g. tuberculosis by *M. tuberculosis*, but although in general that is true there are quite a number of other causes for disease besides germs. If steps are not taken to keep them at least reasonably free, intestinal parasites, e.g. worms and coccidia, can be a very serious problem when animals are kept in large numbers. External parasites, too, such as mange mites, can be a source of trouble, and while inadequate feeding may lead to a deficiency disease it is more likely to lower the resistance of an animal so that it can be more readily attacked by germs or internal or external parasites.

Except for diseases directly caused by feeding, the other diseases are introduced by new stock, or through contact with disease-contaminated utensils. The following examples indicate what can happen. A rabbit breeder in a small way was persuaded against his better judgment to sell some of his breeding does for immediate use in a laboratory. In order to make up his stock he went to a friend for replacements. Within a few days the rabbits he obtained developed snuffles, a highly infectious disease of rabbits, and in a very short time all his own rabbits had developed this disease. The same thing could have happened had he introduced a diseased buck from an outside source. An almost identical example can be quoted in connection with pseudo-tuberculosis in guinea-pigs, a disease which is infectious but takes longer to develop. In this case the owner purchased the entire stock of guinea-pigs from a person who was giving up keeping them. A few deaths occurred amongst the new stock but caused no alarm until deaths started amongst his own stock, which had up till then been free from fatalities. Post-mortem examinations were then carried out and the presence of pseudo-tuberculosis was confirmed. There are other possible means of introducing disease apart from introducing fresh stock. When containers are used for transporting stock to a laboratory there is always an element of risk of them becoming contaminated with a disease at their destination and infecting the breeder's animals on their return. So far as large animals are concerned, this risk is well recognized and the Diseases of Animals Act requires the immediate disinfection of animal transport lorries before a second consignment of animals is carried. Taking animals to shows is also fraught with the risk of picking up disease by close contact, so also is the lending of male animals for breeding purposes. To be absolutely sure of avoiding

contact with an infectious disease, the breeder should adopt an extremely selfish attitude, not lending his stock or equipment and not accepting females from a neighbour for mating. He should also see that containers are thoroughly disinfected on their return after use; he should isolate all new stock for at least 14 days after their arrival and return them if any show signs of disease; he should also isolate all stock for 14 days at least after they have returned from a show to make sure that they have not contracted a disease while at the show. In spite of these precautions, trouble could still arise from contact, since there are some diseases in which the period between picking up the disease germs and the first signs of illness, the incubation period, is longer than 14 days. Such could be the case with pseudotuberculosis. In other animals, for example hydrophobia or rabies in dogs, the incubation period may be up to 6 weeks, though it is usually within 3 weeks, and in scrapie —a nerve disease of sheep—the incubation period can be as long as several years.

Once a disease has broken out among the small animals, steps should be taken to gain control over it. Of prime importance is the necessity to have the disease diagnosed. For that purpose an arrangement has been made with the veterinary investigation service, units of which cover the whole country, whereby specimens are examined and advice given free of charge. If a highly infectious disease is present, e.g. snuffles in rabbits, typhoid or rather paratyphoid in mice and guinea-pigs, it is invariably best to destroy all other animals in the same pen or cages as the diseased animals. The bedding should be removed and buried or burned. Thorough cleaning and disinfection of all the cages should then be carried out, using any of the common disinfectants, e.g. Izal, Jeyes Fluid, etc., in the strengths recommended, and should further outbreaks occur the procedure should be repeated until the disease is under control. To ease the cleaning of the cages, soaking overnight in water to which washing soda has been added is beneficial. The reason why such disinfection is necessary is that the germs may be voided in very large numbers in the manure as in paratyphoid, and eating food contaminated with infection will reproduce the disease. The same general procedure should be carried out to control outbreaks of most of the other diseases of small animals. Coccidiosis can be controlled by disinfection combined with treatment of affected cases with sulphamezathine or one of the other drugs now recommended for the treatment of coccidiosis. The use of washing soda in the water for cleaning the cages is particularly valuable in this disease, since it

destroys the small egg-like parasite. For outbreaks of mange the measures are as for coccidiosis except that treatment should consist of dusting with D.D.T. or Gammexane powders followed by cleaning and disinfection.

Following these precautions the last loop-hole for the introduction of disease is the possibility of wild animals—usually rats and mice. This does not apply to birds affected with coccidiosis, since the bird type of coccidia affects only birds. Precautions should always be taken, therefore, to see that the animal houses are proof against vermin, and steps should always be in operation to keep down vermin to a minimum.

To summarize, hygiene involves (*a*) the provision of the animals with adequate food and housing, (*b*) the taking of steps to avoid the introduction of disease, (*c*) the early diagnosis of the cause of the disease, (*d*) the application of treatment with, if necessary, the destruction of sick animals, and finally (*e*) the thorough cleaning and disinfection of the infected cages.

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Ectoparasites

1. THE CONTROL OF SKIN PARASITES

M. Eleanor Cammiade

A very high percentage of the animals bred for scientific and laboratory use are infected by one or more parasites. Although the animals are apparently fit, these parasites must, by nature of their way of life, undermine the health of the animal. Not only are animals affected directly by the pests a constant source of danger; they can bring in infection from outside, and they can spread it from one house to another or from one cage to another.

To control parasites more effectively some knowledge of their life-history is of great importance. It is of little use to restrict the control of the bedbug to powdering the animal with an insecticide, because the bug is only to be found on the animal when it is taking its meal of blood, the remainder of the time it is hiding in some tiny crack or crevice in the animal's cage or has gone further afield and is hiding somewhere in the animal house itself. Then, as the bug can go for a year without another meal (if conditions are suitable), the effect of the insecticide will have worn off by the time that the bug returns, feeds and lays its eggs.

Parasites, by definition, "live at the expense of another". They are to be found in very many shapes and forms in the animal kingdom, but this article will deal primarily with those which belong to the two great groups, the Insects (*Insecta*) and the Spiders (*Arachnida*). They are very closely related, being almost without exception land-living and air-breathing as adults, and they lay eggs. They have either hard (brittle) or tough (leathery) outer skins (exoskeletons) and have jointed legs. In the adult stage all insects (bugs, flies, fleas, lice, beetles and moths, etc.) have six legs, whereas the arachnids (mites and ticks) have eight legs.

As in so many cases, prevention is better than cure. Prevention means both keeping an eye on your present stock and making perfectly sure that no infested animal or box enters your houses from outside. It is highly probable that all boxes labelled LIVESTOCK are packed in the same luggage van by the railway authorities, so that parasites can pass from one to another. Wood is the usual material used for travelling-

boxes. This provides an ideal home for many small parasites such as bugs. Therefore the boxes should be sterilized and disinfected as thoroughly as possible after use. Parasites may also come into the animal house in hay, sawdust and even foodstuffs, and therefore constant war has to be waged against them.

The more usual insect and arachnid parasites are listed below, together with a brief description and life-history.

(A) Bedbugs

The adult bedbug is a brown flat wingless insect. It is oval in shape and about 4 mm long by 3 mm broad. Its young resemble miniature adults. As has been stated in the introduction, it lives almost its entire life away from the animal on which it feeds. Bedbugs inhabit cracks and crevices of boxes (particularly wooden ones), loose-fitting joints, nail holes, cracks in the mortar and plaster of the room, behind electric switch plates and wainscoting, under loose-fitting wallpaper and so on. They are very active creatures, and may live at some considerable distance from their host. As they are nocturnal animals they may themselves be overlooked but their presence can be recognized in the house by the characteristic "ink-spot" faeces outside the holes in which they live, by their cast-off papery skins, and by their tiny white eggs. The bugs feed at night, taking only a few minutes over their meal, but within that time they can consume more than their own weight in blood. Their whole life is affected by temperature; they will be more active and complete their life-histories in a much shorter time in hot temperatures. At 23°C (73°F) the female, after pairing, will feed and lay eggs repeatedly. Assuming she has two meals a week she will lay between five and ten eggs a week for 2-5 months. The eggs are pearly oval objects which are cemented to the surface so that they are not easily dislodged. Like most insects the young hatch from the egg and undergo a series of moults before becoming adult.

The control of bugs is best carried out by the destruction of the bugs' home. The animal houses should be in a good state of repair, and no litter should be left about; furniture and apparatus not in use should not be stored in the rooms. The animal cages or runs will provide less shelter if they are of metal rather than of wood and if they are of a comparatively small and simple pattern and can be readily sterilized. In cases of slight infestation, thorough cleaning and scrubbing, together with the spraying of the focal points of the infection with a reliable insecticide (e.g. 0·5% DDT or 10% Lethane), should be

sufficient. Make sure that the spray penetrates the infected crevices. In cases of heavy infestation, drastic measures may be necessary. Fumigation with cyanide fumes is about the only reliable way of clearing up the infestation in one attempt, but its disadvantages are that it is dangerously poisonous and the animals must be kept out of the room for at least two days. The most effective simple measure is to spray the walls and cages with a persistent insecticide, e.g. 0·5% DDT in odourless kerosene. Use a sprayer with the nozzle adjusted to give coarse drops, paying special attention to all crevices which should be flooded with the insecticide. The general wall surface should be covered with a film of about 5 cc ($\frac{1}{16}$ oz) per sq. ft. The insecticide is slow acting, taking 24-36 hours to prove effective, but should remain lethal to bugs for several months if undisturbed. It becomes less effective when covered with dust, and a small amount is lost with each cleaning of the room.

(B) FLEAS

Fleas, unlike bugs, do not usually go from one species of animal to another, e.g. dog fleas are normally not found on animals other than dogs and can be distinguished from all other types of fleas by certain characteristics. But in general appearance all fleas are brown insects, flattened from side to side (to enable them to pass easily between the hairs), and about 2 mm long. Apart from the irritation caused by their bites, fleas are dangerous because they are the carriers of several diseases. Normally these diseases are spread from rat to rat or man to man, but when the flea forsakes its usual host, other animals (including man) will become infected by the disease. Perhaps the most famous example of this is the Great Plague which swept Europe in the mid-seventeenth century, and which still occurs in the East, rats being the usual victims of the plague. Murine typhus is another disease usually spread by fleas from rat to rat, and on occasion to man. Many tape-worms are carried (in one stage of their life-history) by fleas.

After mating, the female flea lays its eggs in the fur or plumage of the host. The eggs fall to the ground usually near the host's sleeping-place and there they develop into tiny maggot-like larvae which live among the dirt and rubbish. They live on organic debris, particularly on the partly digested blood in the adult flea's droppings. Then, like the caterpillar, the larva spins a cocoon inside which it becomes a chrysalis or pupa, and it can remain in this state for a very long time, although in warm weather the time taken from egg to adult may only

be three or four weeks. The return of the host to its nest may be a sufficient stimulus to cause the adult flea to emerge from the pupal case.

In warm weather adult fleas live 3-4 months (up to a year in cold temperatures). Adult fleas live on blood and at a warm temperature (23°C or 73°F) they can live only a week or so without food. In very dry air they soon die.

To control fleas two measures must be taken. Firstly, the destruction of the adult flea on the animal's body and, secondly, the destruction of the young stages in the breeding sites. DDT is a very effective insecticide, but many small animals such as rats, mice and canaries, and even larger animals which lick themselves, e.g. cats, are susceptible to DDT and may be killed by it. Pyrethrum powder containing 0·5% pyrethrins, or powdered derris diluted with mineral dust to give 1% of rotenone, or a powder containing 0·5% gamma BHC may be used. Some authorities advocate the dipping of the animals because it is difficult to ensure that the powder insecticide reaches all the areas where parasites may be. If a small area is left unpowdered it becomes the focal point of a new infection. The animals may be dipped in a solution containing 0·25% BHC for guinea-pigs and 0·1% BHC for rats and mice. It should be sufficient if the animal is totally immersed and allowed to remain in the bath for 15 seconds. Treatment should be repeated once after 8 or 10 days as the eggs which were unaffected by the first treatment should have developed sufficiently to be killed by the second treatment. It is claimed that time can be saved by immersing several animals at once in special cages, and that the treatment is more thorough than dusting. So long as the bath is at blood temperature and the animals are allowed to remain in an atmosphere at the appropriate temperature afterwards until they are quite dry, no harmful effects will result from the bath.

To destroy the young stages the room and boxes or hutches should be cleaned and all combustible rubbish burnt. The bottom should be swabbed over with water containing 4% lysol. When the floor is dry, a little of the powder insecticide used for treating the animals should be dusted over it, giving particular attention to corners and crevices. If there are any inaccessible places where fleas may breed, flake naphthalene should be sprinkled in them.

(C) Lice

Lice may be divided into two classes: (*a*) blood sucking lice (*Anoplura*)

which are to be found only on mammals, (*b*) lice which bite off particles of skin, scurf or feather (*Mallophaga*) which are usually found on birds. Both types spend their entire life on the host, cementing their eggs to fur or feathers; they usually keep to one species of host and if separated from it die within a few days. Lice are especially common on animals in a weak state of health, e.g. those on deficient diets. The rat louse will only transmit murine typhus. There are four kinds of lice to be found in guinea-pigs and they are unusual in that they belong to the Mallophaga or so-called "bird-louse" group.

Treatment is the same as that for animals with fleas. At the same time it is a good idea to dust the corners of the cages with insecticide.

(D) Ticks

In this country ticks very rarely occur in an animal house. Should they occur (e.g. they are very common on newly caught wild hedgehogs), they can be destroyed by touching them with derris emulsion or an arsenical cattle dip applied with a small brush.

(E) Mange Mites

Mange mites may either live on the skin causing superficial irritation or may enter the skin or its glands and cause some of the conditions known as mange or scab. In the rabbit the commonest form of mange is ear-canker. The infested areas irritate and scratching by the rabbit causes brown scabs. Body mange in which the fur falls from areas of the face, head and roots of the ears is also caused by mites causing intense irritation and the subsequent clawing of the infected areas by the rabbit. Burrowing mites will cause crusty lesions round the eyes and nose. Mites are very common in mice, and in certain circumstances the infestation may be extremely heavy, so that the fur becomes thin and patchy.

The life-history of these mites is not known in detail.

(F) Blood-Sucking Mites

Certain mites suck the blood of mammals and birds. One which is found on rats has a very short life-cycle (12 days). The adult takes a blood meal, leaves its host, resting in a crack of soil or building and lays a number of eggs, repeating this performance after each meal. The eggs hatch and the larva, after feeding on blood, moults. After two moults it is fully adult. This mite not only bites rats but several other rodents and man. The bite is intensely irritating.

Mites are susceptible to preparations containing sulphur, such as

sulphur dusts, lotions and ointments; to benzyl benzoate; and to gamma BHC. Any of these preparations may be applied locally to the infected animal. Alternatively, dipping (as described under Fleas) in gamma BHC, or dusting, will keep the infestation under control, and may eliminate it altogether.

ACKNOWLEDGEMENTS

Much of the information in this article has been obtained from the chapter entitled "Pests of the Animal House and their Control", by Buxton and Busvine (to whom I acknowledge my indebtedness) in the U.F.A.W. Handbook on the Care and Management of Laboratory Animals. For the rest, which is not thus attributable to them, I accept responsibility.

NOTE

It is invidious to mention any proprietary pesticides by name, for there are many very efficient preparations on the market. But one may be permitted, perhaps, to note that "gamma BHC" is more usually known by its trade name of "Gammexane", which is a common ingredient of many such preparations.

2. MITE ERADICATION

A. A. Tuffery

Laboratory mice are particularly prone to infestation with two species of mites known as *Myobia musculi* and *Mycoptes musculinus*. Small numbers of these mites and their eggs can be found in most stocks of mice, where they exist with very little injury to the animals. Larger numbers can give rise to some loss of condition and the coat may lose some of its sheen. In very bad cases of infestation the skin becomes quite scabby, presumably due to the animals scratching at irritation caused by the mites.

Control of these infestations is relatively easy. It is difficult to get a complete and absolute eradication of mites, but, it is quite easy to reduce the numbers present to insignificant proportions. Four chemicals have been used as miticides with some success in the United Kingdom and the United States. Their use is outlined briefly below.

(A) γ-BHC (GAMMA BENZINE HEXACHLORIDE). COMMERCIAL NAME: GAMMEXANE

While perhaps not quite so efficient as the remaining three miticides

to be listed, Gammexane, or Gammexane-containing preparations, are likely to be the most easily obtained. They are easy to apply and where infestation is not too extensive, will prove to be of considerable value. The powder is simply dusted into the animal's fur very thoroughly and the process needs to be given about twice, or possibly three times, with a fortnight's interval between treatment. This method should not be used regularly but only at long intervals as occasion arises, since it is possible that strains of mites may develop which become resistant to Gammexane.

(B) TETRAETHYLTHIURAM MONOSULPHIDE. COMMERCIAL NAME: TETMOSOL[1]

The stock solution of this substance, as supplied in England, is a 25% alcoholic solution. For use this can be diluted with fourteen parts of water, and the animals are dipped into this solution which is given twice with an interval of a fortnight between each treatment. Some little care needs to be taken with this process—see notes below.

(C) ARAMITE 15W (2-[P-TERT-BUTYL PHENOXY] ISOPROPYL-2-CHLOR-ETHYL SULPHITE)

This chemical is not readily available in England, but can be bought from the Naugatuck Chemical Company, Naugatuck, Connecticut, U.S.A.[2] The Aramite 15W is prepared as a 2% suspension in water which contains a wetting agent (0·1% Nacconal NRSF was used in the original American description of the technique). In England a small quantity of cetramide or other surface active agent can be used for this purpose. The mice are dipped into this solution.

(D) DMC (DI-P-CHLOROPHENYL) METHYLCARBINOL[3]

The DMC is prepared as a 0·2% solution in 50% ethyl alcohol. In making up this solution it is necessary to dissolve the material initially in 95% alcohol and dilute it with distilled water to the required concentration. Mice are treated by dipping.

[1] Obtainable from Imperial Chemical Industries, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire, England.

[2] British agents are U.S. Rubber International (Great Britain) Ltd., 62 and 64 Horseferry Road, London, S.W.1.

[3] Not obtainable in the United Kingdom but supplied by the Sherwin Williams Coy., Insecticide Division, 113 Guildhall Building, Cleveland 1, Ohio, U.S.A.

(E) Notes on Dipping Operations

Dipping mice, even though it sounds a hazardous operation, is, in practise, quite simple and straightforward and few casualties are met with. The solution to be used should be held in a shallow dish, such as a pie dish or bowl, and should be kept warm at blood-heat temperature. The whole operation should be carried out in warm surroundings and the mice after dipping, particularly if the alcoholic DMC solution has been used, should be placed in very warm surroundings until they have dried out and recovered from their sudden enforced swim. Boxes containing such mice can either be placed on, or over, a radiator or in front of an electric fire. Care must be taken that they are not overheated, but they do require to be kept rather warmer than usual. The process should not be carried out in draughts.

When dipping the animals they are simply held by the tail, dropped head first into the solution and allowed to swim to the side of the dish before they are removed. Before they are placed in their drying cages the animals can be held on the table for a moment or two while the excess liquid drains off. Whilst this is being done their mouth, whiskers and eyes can be swabbed carefully with some more of the miticide solution on a piece of cotton-wool. This is to ensure that mites that are frequently found clinging to the base of hairs in this region are properly brought in contact with the chemical.

There is one other important point to be kept in mind. It is obviously quite useless to attempt to demite animals and then place them back into the dirty cage from which they have just come. These cages and bedding are quite capable of holding numbers of mites which will soon find their way back to their host. After dipping, mice should be placed into thoroughly cleaned boxes containing fresh bedding and food materials, etc. The dirty boxes should be scrubbed and cleaned before they are put into use again.

In my experience the dipping technique, using DMC, appears to be the most successful. Dipping with Aramite has not proved so efficient at eliminating mites. All four methods have been used in various laboratories with success.

The commercial mouse breeder would probably find that Gammexane was the most easily obtained miticide and, providing infestation is not allowed to become too extensive, he should probably gain sufficient control using this chemical. For a really thorough treatment of a stock of mice, however, DMC, or possibly Tetmosol, is likely to give the best results.

REFERENCES

Cook, R. (1953). *Brit. vet. J.* **109**, 113-116. Murine Mange: the Control of *Mycoptes musculinus* and *Myobia musculi* infestations.

Flynn, R. J. (1954). *Proc. Animal Care Panel*, 5th Ann. Meeting, 96-104. Mouse Mange.

Flynn, R. J. (1955). *Proc. Animal Care Panel*, 6th Ann. Meeting, 76-91.

Stoner, R. D. and Hale, W. M. (1953). *J. econ. Entomol.* **46**, 692. A method for eradication of the mite *Mycoptes musculinus* from Laboratory mice.

3. RECOGNITION AND CONTROL OF FLIES

G. Porter

Fly population is closely related to sanitary conditions, and large numbers are produced where attractive breeding materials are plentiful and where climatic conditions are favourable. Flies are so proficient in their foraging habits and such prolific breeders that they will undoubtedly breed under the most hygienic conditions but in vastly reduced numbers.

In order to understand fully the methods used for the prevention and destruction of flies, a short life-history is given of the common species found in this country.

(A) The Common House Fly (*Musca domestica*)

This species is the commonest of the British flies and the most difficult to control. The female can lay four batches of eggs per year, each batch containing from 100 to 200 eggs. These eggs are deposited in rotting vegetable or organic matter, manure, food or offal.

The eggs are small, white, sticky and rather elongated. They hatch in 2-4 days depending on the temperature, the most favourable being 18-24°C.

The larva is cream coloured and tapers to one end, it is very active and burrows quickly beneath the surface in order to avoid strong light. It lives on the rotting matter on which it feeds until it becomes fully grown within approximately five days. When it pupates the skin hardens and becomes gradually darker in colour until it is almost black. The young fly emerges and pushes its way to the surface, where it spends the first few hours of its life briskly running about until the wings expand and dry.

(B) The Lesser House Fly (*Fannia canicularis*)

The vast majority of flies found in domestic houses in Britain during

the early summer belong to this species. It usually breeds in rotting vegetables, but a favourite breeding ground is animal excreta. The larva is a dirty off-white colour, and possesses a tassel-like appendage from each segment of the body. The details of development are similar to the common house fly.

Since these insects breed on decaying vegetables it is possible for the larva to find their way into the intestines of the animal causing intestinal infection.

(C) The Stable Fly (*Stomoxys calcitrans*)

The stable fly rather resembles the house fly, but it has a spotted abdomen and is a blood sucker, being furnished with a strong sucking organ (proboscis). It commonly attacks animals, drawing red beads at every thrust, it also bites humans (it has been said to carry the virus of poliomyelitis) and probably acts as a mechanical carrier of many diseases.

(D) Carcase Flies

Under this heading two species are to be considered: "bluebottles" (*Calliphora*), and "greenbottles" (*Chrysomyia*). The female bluebottles and greenbottles deposit a large number of white cigar-shaped eggs on decomposing material, preferably a carcase, and these hatch very rapidly to small active cream coloured maggots. The maggots feed by means of two powerful claws situated within the mouth, which can be protruded at will and are able to tear and consume animal tissues. With an ample supply of food and favourable climatic conditions the maggots attain full growth in a matter of days, then if possible burrow in the earth or decaying matter, emerging as flies some day later.

These insects are sometimes considered harmless, but should the eggs be laid in the natural orifices of an animal's body the maggots attack the living tissue causing great damage often with fatal results.

(E) Midges (*Chironomidae*)

Midges are fairly common in most parts of this country at certain times of the year. The population of these insects is controlled by the geographical position and the botanical facilities available. In damp mossy ground and where the furze and undergrowth is thick, they are multitudinous. They require a high temperature and a high relative humidity for reproduction, and the eggs are laid under damp stone walls, mossy banks and the damp crevices and cracks along the banks

of open ditches. Larvae hatch out in about 9-12 days, and shelter, darkness and moist organic material are essential for their growth.

(F) Mosquitoes (*Culicidae*)

Mosquitoes are distinguished by the pattern of veins and scales on their wings, but it is true to say that different species of this tribe do vary and some have spotted wings.

The female alone sucks blood and after a meal she rests to digest it while her eggs rapidly mature in her ovaries. In due course she makes for water, where she lays cigar-shaped eggs; after a few days the minute larvae hatch; they are termed "darters", and owing to their transparency and size are difficult to see. The larvae grow rapidly, but the time spent in the larval stage is partly controlled by food supply and temperature. Where conditions are favourable, this only takes 7-10 days; the insect then casts its skin and remains in the pupal condition for approximately one week, after which the fully developed insect emerges.

(G) Methods of Control

1. prevention

Removal of all breeding places; for example, the proper disposal by burning or burying of all waste manure, excreta, organic refuse and rotting vegetables. The covering of all foodstuffs. The thorough cleaning of all external drains. The cutting and burning of all surplus undergrowth in the immediate vicinity. The sealing of all cracks on walls and eaves. The flyproofing of all inlets such as doors, windows and ventilators.

2. destruction

Unfortunately it is not possible to remove all potential breeding places, therefore a method of control and destruction must be reverted to. Against adult flies, DDT has a powerful lethal action but unfortunately no repellant action, and it does not prevent them laying eggs, nor does it affect the eggs, and as the larvae burrow under the surface it does not affect pupation or kill the pupae. A liberal and continual spraying of the surface should kill the young flies when they come to the surface in their crawler stage. The object, therefore, should be to cover the surface and approximately six feet surrounding with a residual film of DDT sufficient to kill flies alighting on the surface or emerging from the pupal stage. The required dose is 100 mg of DDT per sq. ft of surface area; that is, 8 oz of 5% DDT in kerosene for each

100 sq. ft. This treatment is of no value on manure or refuse heaps if there is fresh material being added constantly.

In cases where the fly population is very thick and where one cannot control the breeding places, the walls, ceiling, electric light bulbs and the sides of racks must be treated with DDT or the rooms fitted with an aerosol-generator. The night resting places and the outdoor resting places must not be overlooked, i.e. electric-light cords, window surroundings, outside walls which face the sun, incinerators and both sides of the outer door. Where gauze is used to protect open windows or ventilators, this should be wrung through kerosene containing 5% DDT.

The sodium arsenite trap is a metal tray with an arched piece of tin covered with gauze kept moist with the sodium arsenite solution in the tray. These traps are most effective in dry weather when the insects are seeking moisture. The solution is highly poisonous to man and animals and must be kept well out of the reach of animals and children. It is composed of 1-2% sodium arsenite dissolved in 10% sugar in water.

3. ELECTRIC FUMIGATOR

This apparatus resembles an electric-light bulb with two small apertures for dropping in special fumigating tablets. It is not necessary to have the room completely sealed, but the fumes can only travel a certain distance. Microscopic crystals will form on all solid surfaces within the range of the fumigator. This fumigator is non-poisonous, is completely harmless to man and animals, and the fumes do not affect food.

One 40-watt bulb will successfully fumigate a room of approximately 150-200 cu. ft.

It has been recommended that this fumigator can be used in the fight against myxomatosis-carrying insects by ensuring that the crystals are allowed to fall on the rabbit fur. This is achieved by allowing a fumigator to operate over the animals for approximately one hour. Where the hutches are the outdoor type they should be completely covered with hessian and a fumigator allowed to operate within the enclosure for approximately one hour. The animals are not removed as the crystals then form on the rabbit fur. After one hour's fumigation, ventilate in the normal way. This method should provide complete immunity from any form of insect for approximately 10-20 days. Attempts should not be made to extend the period of fumigation in order to obtain longer periods of immunity.

4. PROTECTION OF OUTDOOR COLONIES

Where the animals are housed in outdoor hutches, the following suggestions should be considered.

(*a*) Daily cleaning and thorough sweeping around the hutches; the ground can be sprayed only if no animal or foodstuff is likely to come in contact with it.

(*b*) Light wooden frames covered with hessian and made large enough to cover completely all openings in the hutches will exclude all insects.

(*c*) All green foodstuffs to be completely covered by hessian, all other materials kept indoors in a flyproof room.

(*d*) The use of the electric fumigator which fits into the normal electric-light fitting.

Not only the health of your rabbits but the health of your family and all other animals depend to a large extent on the control of these insects during the spring and summer months.

10

Animals in Research

1. THE USE OF ANIMALS IN EXPERIMENTAL SCIENCE

W. Lane-Petter

The experimental method is now accepted as the correct approach to scientific knowledge, and consists of the intricate study of what has been proved—the very word "prove" means to test—the deduction therefrom that something not yet established may be true, and the testing of that deduction by experiment or trial.

This method is applied to great effect in the enormous field of biology, which embraces everything that is related to the mysterious process called Life, of whose fundamental character so little is known even yet. Within the bodies of all the higher animals, to take a simple example, is a pair of organs called kidneys. These have blood vessels leading to and from them, and each has also a tube (ureter) joining it to the bladder, from which another tube (urethra) goes to the surface of the body, where it discharges the waste matter called urine.

Since the blood vessels carry only blood, and no other tube reaches the kidney, it is clear that urine is made either in the kidney or in the bladder, or possibly in both. Let us suppose that an investigator wished to find out where urine is made. By means of a delicate instrument he passes a fine flexible tube through the urinary orifice into the bladder and thence along the ureter and right up to the kidney. A fluid is now seen to drip from the free end of the tube (catheter) and this fluid is found to be indistinguishable from urine; it is, in fact, urine. This shows him that urine comes from, and may fairly be assumed to be made in, the kidney, though it is possible that the bladder also makes urine.

To settle this point, the investigator may now take an animal, render it incapable of feeling pain, and then cut it open, detach the ureters from the bladder and bring them to the surface of the body. He now finds that urine flows from the ureters but does not form in the bladder, no matter how long he may keep the animal alive, whether conscious or unconscious. This provides him with a working assumption: that urine is made only in the kidney, and he may be content to regard this as true, but only so long as it remains without disproof; it might possibly be that the bladder does make urine, but only when

stimulated by urine coming from the kidney, or perhaps by nervous impulses coming through nerves that he has unwittingly cut when he severed the ureters. To test this possibility, he might devise some means of measuring the rate of flow from each ureter and that from the bladder. And so experimental science goes on, step by step, constantly struggling towards the truth, always thoughtful in its approach, careful in its method and humble in its assertions.

This is only one elementary example of only one way of seeking an answer to only one question of biological fact: there is no limit to what man does not yet know, but equally no limit to his study, ingenuity and persistence in seeking to learn.

Another, and a totally different, kind of experiment commonly carried out on an animal is done when some sufferer, human or animal —for it must be appreciated that the diseases from which animals suffer are studied in exactly the same way as those of man—cannot be medically treated with complete confidence because some doubt exists as to the nature of the malady. Such a case is that of pleurisy in man. In this condition the inflamed pleural membranes pour out quantities of fluid in an attempt to dispose of the intensely painful friction that the inflammation causes. Now one of the most important causes of pleurisy is tuberculosis, in which case there are always present in the pleural fluid the minute organisms (mycobacteria) that cause tuberculosis. So some of this fluid is taken from the patient by means of a long hollow needle and injected into a guinea-pig. From 3 to 6 weeks later the animal is painlessly killed and its body opened, whereupon, if the patient was, in fact, suffering from tubercular pleurisy, the characteristic signs of tuberculosis will be found in the guinea-pig, thereby making or confirming the diagnosis of tuberculosis in the patient much sooner than it would have been had the physician merely waited for more definite signs of development, and the sooner the diagnosis is made, the better the chance of successful treatment and recovery.

Manufacturers of preparations intended to be injected into patients, such as infusion fluid or glucose saline, have been gravely troubled by the fact that, despite their most careful and costly precautions, there occasionally appears among their products a batch that, on injection, causes fever in the patient. This is always disturbing (though not always important), for no manufacturer can tolerate doubts about the harmlessness of his products.

This fever is caused by the presence in the injected material of

exceedingly minute amounts of what is probably the most powerful substance, in bodily effect, in existence. It is known as "pyrogen" (heat-maker), and is so powerful that a millionth part of a gram will cause a rise of body temperature. It is believed to be an end-product of bacterial life, but unlike living bacteria it is unaffected by heat, so that sterilization does not destroy it. Batches of material containing an appreciable amount of pyrogen are useless for injection and must be thrown away, but how to discover the existence of quantities a thousand times too small to be capable of detection by chemical analysis or physical observation?

To do this, a rabbit is set in a box with its head comfortably projecting through a hole in front. A hair-like kind of electric thermometer (thermocouple) is inserted into its back passage and an apparatus set up whereby any change in the animal's temperature is recorded on a moving band of paper. The animal is now injected with what should be a completely harmless dose of the pharmaceutical product. If pyrogen is present, the electrically recorded temperature-line on the paper slopes upwards, and the batch of the product from which that sample was taken must be thrown away. If the rabbit's temperature does not rise significantly, there is no pyrogen present (or not enough to matter) and the product may safely be supplied for use as intended.

These three experiments have been briefly described as samples of the three main classes of experiments on living animals: those that aim at increasing knowledge of the working of the body, those that are more individually directed towards knowledge of what is wrong with one particular body, and those that must be done on new substances intended for use in the treatment of disease, etc., before they may be regarded as safe for general use.

It is of course obvious that since a mouse is not a man, an experiment on a mouse will not necessarily give the same answer as a similar one done on a man. But though a mouse is not a man, it is largely made up of kinds of tissue that are very similar to those in man, and consequently a drug that damages, say the tubules of the kidney in a mouse, is likely—but not certain—to damage those in man. Conversely, a drug that produces its desired healing effect in a mouse without causing at the same time any damage stands a good chance of being of equal value in man, and may justifiably be administered to man, starting cautiously with very small doses and gradually increasing them.

No one enjoys having to subject an animal to a painful experiment, and many scientists have submitted themselves to very severe opera-

tions in order to advance knowledge, but certain material considerations must also be taken into account in deciding whether any particular experiment should be done on animals. All laboratory animals breed more quickly and more numerously than man; they can be kept in cages under identical conditions and so protected against influences other than that which is being investigated; they are relatively cheap and easy to feed; and their different constitution makes them respond sooner than man to the influence of drugs, etc.

Our legislation provides that no one shall carry out any painful experiment on an animal unless he has been licensed by the Home Secretary to do exactly that experiment. This permission is given only after investigation and is made subject to a number of conditions concerning, for example, the kind of experiment that may be done, the place where it is to be done, the kind of animal to be used, the degree and duration of suffering allowable, the exact reason for needing to do the experiment on an animal at all, the use of anaesthetics, and other matters, but these cannot be detailed here.

Inspectors appointed by the Home Office visit from time to time all places where such experiments are carried out, and may be consulted for advice in connection with the Cruelty to Animals Act, 1876. Anyone who seriously intends to carry out an experiment under the Act, but is unfamiliar with the way to go about it, should first clear his mind on all the points mentioned above and then write to the Under-Secretary of State, Home Office, Whitehall, London, S.W.1, to ask for advice. But in formulating his intentions he should appreciate that an experimenter under the Act is always expected to do any necessary experiment on an animal that has first been rendered totally incapable of feeling pain and to kill it before it can recover consciousness; if, however, this would frustrate his object, and only then, may he be allowed to permit the animal to survive the experiment.

One primary essential of the experimental method is that in every experiment there shall be, so far as is possible, only one variable. This means, for example, that when a manufacturer, in order to determine at what dosage one of his products begins to be poisonous, injects a large number of mice with a graduated series of doses of the product, he must be able to rely on all his mice being quite healthy and similar in constitution and physique. If this were not so, it is clear that the fact that one mouse showed signs of a poisonous effect with a given dose might be due to its having a lower resistance to the drug than its cage-mates, and so be of no experimental value whatever.

We thus see that whereas the person who buys an animal, as a pet, for the show bench, or for its produce, wants one that is different and better than the average—either for pride of ownership, the winning of prizes, or the value of its produce—the scientific investigator wants his animals to be not only in perfect health but also uniform in all respects, so that he may regard any of them as being exactly replaceable by another of the same kind, sex, age and weight.

2. THE USE OF GUINEA-PIGS IN THE LABORATORY

A. J. H. Tomlinson

On arrival at the laboratory the guinea-pigs are divided according to sex and kept for a few days in quarantine. This is to give them an opportunity to recover from the effects of the journey and to get used to the different conditions in the laboratory animal house. During their life in the laboratory the animals receive the best of care and attention, but a change of management and surroundings affects all livestock adversely for the first few days. Animals are usually used in rotation and the majority receive an inoculation by a syringe and needle. This causes no more upset than is experienced by the average recruit when he receives his inoculations at the hand of military authorities.

Guinea-pigs are used in laboratories in a large number of different ways. The largest number are used for diagnostic purposes, that is to say for the diagnosis of certain infectious diseases in man and animals and for the demonstration of disease-producing bacteria in foodstuffs and industrial raw materials. The great majority of diagnostic tests are made in laboratories by cultural methods that depend on the growth of the disease-producing bacterium on culture media; the cost of the media for one of these tests is only trivial and generally only about one-hundredth of the cost of an examination involving the use of a guinea-pig. There is naturally, therefore, a tendency for laboratory workers to try to develop cultural techniques to eliminate the use of guinea-pigs in diagnostic and other work, particularly if they are unable to obtain satisfactory animals.

A large number of guinea-pigs is used for the diagnosis of tuberculosis in man and animals. From certain types of material the diagnosis can be made quite satisfactorily by cultural methods, but if the number of tubercle bacilli is small and large numbers of other bacteria are present, the best results are obtained by inoculation of a guinea-pig.

Guinea-pigs, after inoculation with a few tubercle bacilli, develop a well-marked tuberculosis that ultimately is fatal. Generally the guinea-pig is killed about 6 weeks after the inoculation and a *post-mortem* examination is carried out to determine whether or not tuberculous lesions are present. At this time the tuberculous process is in its early stages and causes no disability to the animal, and it is impossible to tell from an external examination of the animal whether it is diseased or not.

If the guinea-pig should die before the 6 weeks has elapsed no result is obtained from the test and it has to be repeated. Often this is not possible for the original material may have been obtained at an operation on a hospital patient: on other occasions it is inconvenient and expensive to obtain a further specimen for test, as when a veterinary surgeon has to visit a farm twenty miles from his surgery. The whole success of this method of diagnosis of tuberculosis depends on having as few failures as possible. With good animals less than 1% of inoculated animals die before the end of the 6-week period: under other circumstances 20-40% of inoculated animals may die. The causes of these premature deaths are varied, but most of them can be related to the use of undersized ill-conditioned animals, to the use of animals suffering from chronic infection, or to the epidemic spread of an acute infection such as that caused by organisms of the *Salmonella* group. Although the inoculation of the usual materials causes little upset in healthy animals, the inoculation of similar materials or even of entirely innocuous substances like water, in unhealthy animals may be fatal within a few days.

Similar considerations apply to the other diagnostic tests for which guinea-pigs are used; material is inoculated and the animal is carefully watched for a period that depends on the type of infection suspected. Often the animal shows so few symptoms that the only way of telling if it is infected is by taking its temperature several times a day. Again animals that die from some intercurrent disease represent wasted material and an unsuccessful test, and animals that are unhealthy to start with are unlikely to give reliable temperature readings.

The serum from the blood of guinea-pigs is an important reagent in a number of laboratory tests. To provide this adult guinea-pigs are kept and used as regular blood donors, suffering about as much inconvenience as their human equivalents. Again healthy animals are essential since only healthy animals can make good the repeated small blood loss: the serum from unhealthy animals is often inferior in quality and may give entirely false results in some tests.

Another type of test for which the guinea-pig is very suitable is the standardization of toxins and antisera. Antisera have to be standardized so that known amounts can be inoculated for the prevention or cure of infections in man and animals. Very often the animal has to endure the indignity of being shaved so that small reactions can be observed in the skin. Animals in good health and free from all infections can only be used for this purpose. Poor-quality animals give unreliable or even false results. Guinea-pigs are also used in tests to determine the toxicity and efficiency of new drugs, in nutritional experiments and in many other ways, but in each case the reliability of the results depends on the quality of the animals used.

In conclusion it will be seen that the guinea-pig can be used for a wide range of laboratory investigations covering practically the whole field of medical science. In each case the success or failure of the investigation depends very largely on the quality and health of the animals used, and it is certain that the breeder who can consistently and regularly provide first-class animals for the laboratory worker is making a real contribution to the health of the community.

3. THE USE OF ANIMALS IN THE DIAGNOSIS OF PREGNANCY

W. W. Walther

Pregnancy tests are as old as history. It is said that the ancient Egyptians had such a test in which two batches of corn were sown, one being watered with the urine of the woman who thought she might be pregnant and the other with that of her husband. After a suitable interval the shoots of the two batches of corn were measured and if the wife's batch were longer she was considered to be pregnant.

Today we also use urine as the test substance because quite clearly early on in pregnancy, sometimes within about three weeks of conception, a substance is excreted in the urine of the pregnant woman which, when injected into the circulation of a suitable female animal, will produce changes in its ovaries. These changes consist of little blood spots which can be readily seen on the ovaries by the naked eye. None of the tests is 100% accurate, but nevertheless a high degree of accuracy can be obtained under ideal conditions. Now, these changes also take place when the animal has recently been in association with a male, and for that reason, in order to secure ideal conditions, the test animals

need to be segregated for a certain minimum time before the test is carried out.

In the Aschheim-Zondek method, five mice are needed for each test, and these must be 21-day-old females weighing between 8 and 10 g. Alternatively, single female rats may be used between 30 and 45 days old and weighing not more than 65 g.

I believe that a greater degree of accuracy can be obtained with the Friedman test. The conditions originally laid down for this test were that female rabbits not less than 17 weeks old should be used. The young rabbits are separated at weaning and kept in individual cages for 3-4 weeks before use. The reason for this is that even young does may "jump" each other if kept together in the same cage and thus induce changes similar to those induced by the injection of pregnancy urine. Some textbooks recommend female rabbits "not less than twelve weeks old." These are, of course, cheaper, but a little less reliable as one sometimes gets a young doe with infantile genitalia which will persistently give negative results. To overcome this disadvantage, some authors have used mature non-pregnant does less than 6 months old and weighing not less than 2·5 kg (5½ lb). They should have had one litter and have been isolated for 3-4 weeks before the test.

For guidance of pathologists who may wish to use rabbits for the pregnancy test, it would be useful if breeders would quote prices for the following categories:

(*a*) Virgin does not less than 12 weeks old.
(*b*) Virgin does not less than 17 weeks old.
(*c*) Mature non-pregnant does over 6 months old and over 2·5 kg (5½ lb) which have had one litter.

Since all these animals have to be segregated for 3-4 weeks before they can be used, it would be very helpful if a note were put on the crate in which they are sent stating the length of time they have already been isolated.

More recently various species of toads have been used for pregnancy tests. The female South African horned toad (*Xenopus laevis*) will lay eggs only when stimulated by the male or after injection under the skin of pregnancy urine, and this test is said to be reliable. Unfortunately, it is stated that the best results are only obtained if the toads are bred in South Africa and imported direct, although recent experience does not confirm this contention.

One of the latest modifications is to use the common indigenous toad (*Bufo bufo*). In this case the male is used and he reacts to the

injection of pregnancy urine by emitting a few sperms, but since these have to be demonstrated under the microscope in material collected from the vent by means of a fine glass pipette, the method is not as simple as it sounds. Nevertheless, it is very quick and reliable.

I hope that these few notes will be useful to breeders in helping them to supply the right type of animal thus ensuring more efficient results.

4. THE ROLE OF THE LABORATORY ANIMAL AT A VETERINARY INVESTIGATION CENTRE

James Milne

It is well known amongst breeders that their animals play a very important part in research and diagnosis of disease in the medical world, but it may not be so widely known that small animals are also commonly used in research and diagnosis of certain diseases in the veterinary field.

As our laboratory is a comparatively small unit we do not keep a large stock of small animals nor do we use them for a wide variety of purposes. This short article is an attempt to give the breeder some idea of the importance of the small animal in this branch of veterinary work.

Disease in farm animals costs the country millions of pounds annually apart from the loss of meat for human consumption. The loss would be considerably greater were it not for the small animal. It is used mainly for the diagnosis of disease so that the appropriate vaccine, serum, antibiotic or other drug may be applied in the treatment and prevention of the disease.

The diagnosis of some diseases in sheep depends on the laboratory animal, particularly mice. At this centre we investigate the cause of death in many outbreaks of disease in sheep and find the mouse our most useful animal. Rabbits and guinea-pigs are of secondary importance.

Lamb dysentery and pulpy kidney disease of lambs, and enterotoxaemia of older animals account for many losses. These diseases are caused by an intestinal infection with a germ of the *Clostridium welchii* group. The germ produces a toxin (poison) in the stomach and intestine of the animal and causes its death. If any of the above diseases are suspected, some of the contents of the stomach and intestine are removed from the dead animal, centrifuged and filtered. A small amount of the resulting clear fluid is injected into the blood stream of mice through a tail vein. If the mice die within 24 hours it indicates

that a toxin has probably been present in the intestine. Different types of toxins are associated with these diseases so that further tests must be carried out to discover which one it is. A further batch of mice is injected by way of the blood stream using the filtered intestinal contents neutralized with anti-toxins of types B, C and D. The group which survives the infection indicates the toxin which has been neutralized and the one which has caused the disease. The veterinary surgeon is then advised and can inoculate the remainder of the flock with the appropriate vaccine or serum to prevent further losses.

Another disease of sheep in which the mouse plays its part, although perhaps not such a vital part as that of toxin examination, just mentioned, is pneumonia. One form of pneumonia is caused by a germ (*Pasteurella haemolytica*) which is fatal to mice. (It has been suggested that a virus is the real cause and pasteurellosis a secondary infection.) The germs are recovered from the sheep's lungs by growing them on media (a special jelly substance). They are then inoculated under the skin of a mouse. If the mouse dies, usually within 3 days, the blood is examined with the aid of a microscope and the germs can be readily recognized by their peculiar appearance. They do not have this peculiar appearance when examined directly from the media.

Braxy is a disease of sheep which is caused by another of the Clostridium group, namely *Clostridium septique*. The germ is fatal for guinea-pigs. It attacks the fourth stomach of the sheep where it multiplies rapidly producing a toxin which kills the animal. The germ is isolated from the stomach and grown on media. It is then inoculated into a guinea-pig. If the guinea-pig dies, microscope films are made from the liver and examined when the typical appearance of the germ, together with some other tests, will confirm the disease.

One more disease of sheep in which the laboratory animal is helpful in diagnosis is Listerellosis. This disease affects the brain and the sheep may go blind or walk in circles. The germ is recovered from the brain by growing it on media or by injecting brain material into a rabbit. It does not kill the rabbit but causes an increase in certain white blood cells (monocytes) so that by examining the rabbit's blood daily for a few days before and after injection Listerellosis can be recognized.

Many hospitals and research institutes keep hundreds of guinea-pigs, but we have only a small number here and use them mainly for the confirmation of the presence or absence of tuberculosis in milk, guinea-pigs being very susceptible to tuberculosis. Milk is first examined under the microscope, but if no tubercle germs are seen it does not necessarily

mean that the milk is free from them. A little of the suspected milk is inoculated into the two guinea-pigs, and if they do not die they are killed at 6 and 8 weeks respectively. If tuberculosis is found, the cow from which the milk was obtained has to be destroyed.

Rabbits, like guinea-pigs, are not used a great deal here, but we used a fair number for a time during investigations into myxomatosis. They were infected with the myxomatosis virus and then let loose amongst the wild rabbits on an island off the west coast of Scotland in the hope that the disease would spread. Its failure to spread could only be explained by the lack of biting insects and it was then that further investigations showed that the rabbit flea would spread the disease. The course and spread of the disease in the wild rabbits was carefully observed.

While these are the main purposes for which we employ small animals at this veterinary investigation centre, other conditions arise from time to time which necessitate their use. However, breeders may have been given sufficient indication that their products play a vital part in the diagnosis of diseases in farm animals in this country.

5. THE LAW RELATING TO EXPERIMENTS ON ANIMALS IN GREAT BRITAIN[1]

(A) Scope of the Act

Act 39 & 40 *Vict., Ch.* 77, usually referred to as *The Cruelty to Animals Act* (1876), regulates the use of animals for experiment. It is administered in England, Scotland and Wales by the Home Secretary. Identical or similar legislation is in force in Northern Ireland and the Isle of Man, and in certain other places. Licences granted by the Home Secretary and those granted elsewhere are not interchangeable.

(B) General Principles

"The Cruelty to Animals Act, 1876, imposes restrictions which apply to any experiment calculated to inflict pain upon a living vertebrate animal" (see Return of Experiments, issued each year by the Home Office as a White Paper). Except subject to these restrictions, such experiments are prohibited by the Act. The Act was based on the recommendations of a Royal Commission which was appointed in 1875.

[1] This abstract published by kind permission of The Research Defence Society, 11 Chandos Street, London, W.1.

(C) PAIN

A further Royal Commission was appointed in 1906 and sat for 6 years. It took the view that even the pursuit of knowledge must recognize a limit to the pain which shall be inflicted on an experimental animal, but that it would be inconsistent and unreasonable to impose a greater restriction upon the infliction of pain for the advancement of knowledge than public opinion sanctions in the pursuit of sport, in carrying out such operations as castration and spaying, or in the destruction of rabbits and of rats and other vermin by traps and painful poisons (see Final Report of the Royal Commission on Vivisection, published 1912, p. 64). The Home Secretary gives effect to this view by imposing a pain condition to be observed in all experiments on the conscious animal. This condition requires the licensee to kill an animal which is suffering severe or enduring pain, provided the main result of the experiment has been attained; but if the pain is both severe and enduring, then the animal must in all cases be painlessly killed forthwith.

(D) INSPECTION

All laboratories where animals are used in experiments under the Act of 1876 are inspected at intervals and without notice.

(E) PROSECUTION

Section 21 of the Act states: "A prosecution under this Act against a licensed person shall not be instituted except with the assent in writing of the Secretary of State." It is doubtful if the Home Secretary has ever given this permission. The effect of this Section is to protect the licensee from irresponsible or malicious prosecutions.

(F) REGISTRATION OF PREMISES

The places where experiments under the Act are to be carried out are normally registered by the Home Secretary.

(G) LICENCE

A licence to carry out experiments is granted by the Home Secretary. When the licence is used by itself, every experiment so made is subject to certain restrictions, among which are the following.

(*a*) The animal must be under the influence of an anaesthetic throughout the experiment.

(*b*) The animal must be killed at the end of the experiment while still under the anaesthetic.

(*c*) The experiment must be for the advancement by new discovery of physiological knowledge which will be useful for saving or prolonging life or alleviating suffering.

(H) Certificates

Certificates are given by a president of one of a number of learned bodies, and a professor of a main branch of medical science. The main purpose of certificates generally is to release the licensee from one of the above restrictions. Thus Certificate A releases the licensee from restriction (*a*) above; Certificate B from (*b*) and Certificate C from (*c*).

(I) Advisory Committee

In a small minority of cases, when the Home Secretary is in doubt as to whether or not he should grant a licence or allow a certificate, he may refer the matter to an Advisory Committee. This Committee was set up on the recommendation of the Royal Commission (1906-12). The members are selected from a panel of names submitted by the Royal Society, the Royal College of Physicians and the Royal College of Surgeons, three members from each body: one representative of the Royal College of Veterinary Surgeons and a judge of the High Court occupies the Chair.

(J) Legal Requirements

Licences and certificates are legal documents. They are personal to the holder, and delegation of authority under them is expressly forbidden, whether or not in his presence.

The Home Office looks to the licensee to give strict observance to the relevant extracts of the Act which accompany the licence; to the conditions attached thereto; and to the wording of the certificates which admits of no latitude. Infringement may lead, and in some cases has led, to revocation of the licence. Action may also be taken against the laboratory authority which is responsible for the registered place in which the experiments are carried out. At the time of registration it is stated in a letter that "the Secretary of State relies upon the co-operation of the laboratory authorities in requiring the strict observance within the registered premises of all the provisions of the Act" and if he subsequently considers that his reliance has been misplaced, he may reasonably be expected to take appropriate action, up to and including cancellation of registration. From neither of these decisions is there any appeal.

(K) TEACHING

The Royal Commission of 1906-12 supported the absolute prohibition of experiments on conscious animals in illustration of lectures. There can be no objection, however, to allowing suitable persons to witness experiments performed in accordance with the provisions of the Act, whether under licence alone, or under licence and any certificate.

(L) CINEMATOGRAPH FILMS

Section 6 of the Act says: "Any exhibition to the general public, whether admitted on payment of money or gratuitously, of experiments on living animals calculated to give pain shall be illegal." There is nothing to prevent the making of a cinematographic record of an experiment performed in accordance with the requirements of the Act.

(M) PREGNANCY TESTS

It is questionable whether pregnancy tests using toads, mice, rats or rabbits come under the Act at all, the crux being whether they are "calculated to cause pain". Only a Court of Law can determine such questions and in the absence of a decision in a particular case the licensee must decide the question for himself. Generally speaking, however, it would appear quite unnecessary for pregnancy tests on cold-blooded animals to require a licence, and the same probably applies to the Aschheim-Zondek test. With regard to the Friedman test in its various forms, details of technique may well lead different workers to opposite conclusions.

(N) CONCLUSION

All possibly painful experiments on living vertebrate animals carried out in this country are thus strictly controlled by law, and one may feel satisfied that, when it is necessary to use animals in this way for the advancement of medical and scientific knowledge or for the relief of human suffering every precaution is taken to see that the animal's suffering is kept to a minimum, if not wholly eliminated.

II

Laboratory Standards

1. LABORATORY ANIMAL STANDARDIZATION IN THE UNITED STATES OF AMERICA

Berton F. Hill

The importance of standardization in experimental animals has long been recognized in the United States. Both the Animal Care Panel, since its foundation in 1947, and the Institute of Laboratory Animal Resources, since its establishment by the National Academy of Sciences in 1952, have followed the progress of the British Scheme of Accreditation with great interest. After discussing the problems inherent in a nationwide accreditation programme for several years, the Governing Board of the Institute agreed on 23 May, 1956 to attempt a plan of breeder accreditation and animal certification. Impetus to this decision was given by the need for large numbers of high quality inbred, hybrid and random-bred mice by the Cancer Chemotherapy National Service Center of the National Institutes of Health and by a proposal adopted at the Institute sponsored Conference on Animal Standardization and Accreditation which urged the development of laboratory animal standards. In order that commercial breeders of research animals might be introduced to the concepts and operation of a standards programme, the Institute held a Conference on the Production of Inbred Mice on 6 August, 1956, at which much valuable data were obtained on which to base a realistic accreditation scheme. Subsequently, a contract agreement was drawn up between the National Institutes of Health and the National Academy of Sciences (acting on behalf of the Institute) providing for the establishment of standards for the production of inbred and random-bred mice. A committee, appointed by the Institute to prepare drafts of standards, consisted of three commercial breeders, a nutritionist, five institutional animal colony directors and two workers in the regulatory field. Members of the Institute staff made site survey visits to breeding farms to obtain information on colony operation, construction and equipment. The result of the staff activity and committee deliberation was the publication (Byerly *et al.*, 1957) of the mouse standards.

MINIMUM STANDARDS FOR THE COMMERCIAL PRODUCTION OF RANDOM-BRED AND INBRED LABORATORY MICE

Purpose and Aim of the Mouse Producers Accreditation Programme

The laboratory mouse is the tool chosen for a vast number and variety of studies aimed at the prevention and cure of human diseases. It is also widely utilized in the testing of innumerable drugs and biological products for their usefulness in alleviating human suffering.

In medical research it is of great importance that the tools used be as uniform and standardized as possible, in order that the results obtained in one laboratory may be repeatable in a different laboratory. Uniformity of the tools used in research also increases the accuracy of the results obtained. In addition, it speeds research by decreasing the number of repetitive experiments needed to ascertain that a particular result is not merely due to chance.

Two types of uniformity must be considered in the case of the laboratory mouse: genetic uniformity and environmental uniformity.

Genetic uniformity may be considered from two different aspects depending on the mating system employed. In the case of the genetically undefined white mouse, commonly produced commercially, consistent variability can be achieved by maintaining all commercial colonies under a system of rigidly randomized matings. This will ensure that all lots of white mice obtainable commercially will be uniformly variable in their genetic constitution. Experiments utilizing these white mice could then be designed on the basis of their uniform genetic variability eliminating the present source of error due to uncertainty of the degree of genetic variability of different lots of mice. It cannot be too strongly emphasized that the uniform genetic variability of random-bred white mice is just as important as the uniform genetic invariability of inbred strains of mice.

The genetic constancy of inbred strains of mice can only be maintained by continuing brother × sister matings, beyond the twentieth generation of such matings, for the entire life of the strain. All animals within a strain should be as closely related as possible, tracing back to only a few ancestors in every generation. The usefulness of different inbred mouse strains arises out of the specifically defined physiological characteristics of each strain. Thus, for example, a specific transplantable tumour will grow only in mice belonging to a specific inbred strain and not in any other strain. Mice of different inbred strains may have different susceptibilities to certain infectious diseases or to cancer formation. The probability that all mice of a specific inbred strain will show the same response to a specific experimental treatment is very high, certainly far higher than that for random-bred mice under similar treatment.

Thus, by utilizing the very low degree of genetic variability of inbred mice, experiments can be designed in which almost all variability in the results can be attributed to the experimental treatment and other environmental conditions.

Environmental uniformity, equally as important as genetic uniformity, is in practice more difficult to attain. Since laboratory mice are produced in different localities by different breeders and under different conditions, it is essentially impossible to produce all mice under environmental uniformity. However, it is both possible and practical to approach this ideal condition. This can be accomplished, for both random-bred and inbred mice, by maintaining the animal colony under optimal conditions of cleanliness, nutrition, temperature and humidity, and prevention of disease. Although definitive experimental studies on the best cage design, nutrition, and other problems of colony management are urgently needed, a begin-

ning towards the establishment of optimal environmental conditions in all commercial laboratory mouse colonies can be made by utilizing the past experience of academic and commercial mouse producers. It is out of this experience that the minimum standards presented herewith have been evolved. It should be noted that these are *minimum* standards. It is assumed that all producers will want to establish practices in their colonies which will enable them to raise mice under optimal conditions which will considerably surpass those outlined in the standards.

As both the producers and the accrediting agency gain experience in the administration of the programme of accreditation of mouse producers, changes in the minimum standards will have to be made. As the standards are presented now, they are not intended to prevent any producer from trying out or adopting practices not specifically mentioned in the standards if these practices are designed to improve the conditions under which the mice are raised. In fact it would be very helpful to the accreditation programme if producers would acquaint the accrediting agency with animal husbandry practices which they find to be superior to those mentioned in the standards.

The sole aim of these standards and of this accreditation programme is to aid the commercial mouse breeder in the production of more uniformly healthy, well-fed mice of known genetic background and variability. Such mice will aid immeasurably in increasing the reliability of experimental results obtained in medical research and will thus be of great benefit to mankind.

To the User of Laboratory Mice

The mice produced by accredited breeders under the conditions required by the standards of accreditation are of superior quality. They will give more dependably uniform experimental results and more uniform bioassay results than mice produced under substandard conditions. This superior quality can only be maintained by employing breeding and husbandry practices, which are more expensive than those previously used. It is therefore impossible for an accredited mouse producer to compete on a price-per-mouse basis with dealers and breeders producing mice of average or inferior quality. Since the mice used in any experiment are a minor cost item, it will behoove every investigator to specify to his purchasing agent that mice are to be obtained only from accredited producers.

To the Producer of Laboratory Mice

All methods of housekeeping, nutrition, disease diagnosis and prevention, genetics, and record-keeping—including those recommended in these standards—used in the commercial production of random-bred and inbred laboratory mice must be approved by the accrediting agency. The accrediting agency may give provisional approval to producers who give assurance of comformity within a definite and reasonable period of time.

Producers who meet these standards will receive an official statement from the accrediting agency which they may use as evidence that their products conform to good production standards. Added cost of adherence to these standards properly may be reflected in price.

FACILITIES AND SANITATION

Facilities, equipment, and sanitary practices shall be designed to provide minimal opportunity for transmission of diseases and parasites from the population in one cage to populations in other cages, and to subsequent populations which may occupy the same cage. Such practices will minimize the opportunity for the trans-

mission of diseases and parasites into the producers' premises and from the producers' premises to the users' laboratories.

SANITIZATION: To make physically clean and to remove and/or destroy agencies injurious to the health of laboratory animals.

STERILIZATION: The act or process of killing all living cells, especially micro-organisms.

CAGING AND EQUIPMENT:

1. Cages and racks shall be made of a smooth, corrosion-resistant, impervious, easily cleaned and sanitized material; e.g., stainless steel, plastic, or glass. If present cages or racks do not conform to these specifications, they shall be replaced by cages and racks of approved material within a period of time to be determined by the accrediting agency in consultation with the individual producer.

2. Minimum cage area, per nursing female (including litters of an average litter size for the colony up to nine in number), shall be 30 sq. in.

For litters of an average litter size for the colony of more than nine, add 3 sq. in. for each additional animal.

Maximum number of mice in any cage of any size shall be thirty.

3. Water bottles or other devices, equivalent in protection of water against contamination and approved by the accrediting agency, shall be provided.

4. Feed hoppers constructed of corrosion-resistant material shall be suspended in each cage. All hoppers should be covered or should be so constructed as to prevent entry of mice. They should extend low enough to be reached by pre-weanling mice.

5. Animal-room floors, walls, and ceilings should be made of a smooth, hard, impervious material that can be cleaned easily. New buildings should be constructed of materials other than wood.

BEDDING:

1. Bedding shall consist of sawdust, shavings, or other material not readily eaten. Hay, straw, or shredded sugar cane shall not be used.

2. Bedding shall be protected from exposure to vermin prior to use. It should not be obtained from any unprotected storage source.

3. Bedding shall be dry when received.

4. Storage at the mouse breeder's establishment shall be under clean, cry, vermin-free conditions.

5. Bedding shall be changed weekly or oftener.

BUILDINGS:

1. Animal room temperature should be maintained between 70°F and 80°F. In the summer, buildings can be cooled by painting the roofs with aluminium paint, attaching sprinkler hose to roof, and using ventillating fans inside the building. Air conditioning is recommended.

2. Adequate, draft-free ventilation shall be provided.

3. Buildings shall be rodent-proof and vermin-free.

CLEANING:

1. Animal house floors shall be scrubbed with hot water and detergent once each

week. Walls and ceilings shall be cleaned frequently, preferably by scrubbing with hot water and detergent.

2. Corridors and animal room floors shall be swept daily with a sweeping compound. Frequent dusting is recommended.

3. Cages, feed hoppers, water bottles and watering tubes, or other watering devices shall be very thoroughly cleaned with hot water and detergent or otherwise sanitized before new animals are placed in the cages.

a. All cleaning and sanitization methods used shall be examined for adequacy by the accrediting agency.

b. Mechanical cage washing machines (operating at 180°F) used with an effective detergent are recommended. If these are used, no separate sanitization of cages is necessary under normal conditions.

c. It is recommended that all cages and watering devices be sanitized at least once each week.

4. The water bottle or other watering device removed from any cage shall be returned to the *same* cage unless it is sanitized in a manner approved by the accrediting agency. This is very important for the control of diseases.

5. All refuse and dead animals shall be placed in closed containers and removed from the premises daily. Incineration is recommended.

6. Refuse containers and covers shall be sanitized thoroughly after use and before being brought into the animal room.

DISEASE DIAGNOSIS AND PREVENTION

The introduction of new stock to any breeding colony must always be considered a hazard, and every breeder should use every precaution to protect his own stock. Each inbred nucleus colony will practise the strictest sanitary and disease-prevention measures and every effort will be made to deliver the breeding stock in an uncontaminated and disease-free condition.

1. All breeding stock obtained from any source, including the inbred nucleus, shall be quarantined upon receipt for at least 3 weeks in a room or building isolated from the rest of the mouse colony. It is recommended that during this period samples of these animals be tested for salmonellosis.

2. All persons entering the colony shall wash themselves very thoroughly, and dress in special shoes or clean shoe covers, and clean clothing or uniforms before beginning work in the animal rooms. The clothing, uniforms, shoes and shoe covers are to be worn only in the animal rooms and are not to be removed from the animal room until they are removed for laundering. The clothing, uniforms, and shoe covers shall be washed at least once a week.

3. Personnel handling animals should wash their hands thoroughly at frequent intervals. Hands should be washed immediately after handling sick animals.

4. Visitors should not be allowed in any animal room.

5. To prevent disease dissemination all mice shall be shipped and delivered in new and non-returnable containers which have been approved by the accrediting agency. These containers shall be stored, prior to use, in clean quarters separate from the animal rooms.

6. Periodic examination of samples of mice, taken from different cages in the

colony, shall be made by competent diagnostic laboratories. A minimum of two examinations a year shall be performed at least 4 months apart. These examinations may include the following diseases for which reports shall be submitted to the accrediting agency and to the producer by the diagnostic laboratory. Other examinations may be made at the request of the producer or upon request of users. The diagnostic laboratory shall be approved by the accrediting agency.

a. Salmonellosis
b. Ectromelia
c. Other pathological conditions found
  1. No evidence of.....since.....
  2. Incidence of.....:.....% during preceding 6 months or 1 year.

7. Vaccination against ectromelia of all breeding and future breeding animals is recommended. If this procedure is followed, the requirement for periodic examinations for ectromelia of samples of mice from the colony may be waived by the accrediting agency.

8. Standard procedures for submitting materials for diagnosis, for the handling of sick animals, and for the control of epidemics shall be issued by the diagnostic laboratories and approved by the accrediting agency.

9. Proper records should be kept by each breeder to enable him to trace mortality patterns in the mouse colony.

GENETICS AND RECORD KEEPING

Mice distributed by accredited producers shall be identified with respect to ancestry and method of mating used in their production. Such mice may be certified by the accrediting agency.

Except in instances in which specific genetic or health requirements necessitate the introduction of new stock, maintenance of a closed population with no introduction of outside stock by each producer generally improves the uniformity of the stock and facilitates the maintenance of health in the colony.

The keeping of production records for each mating or breeding pen is recommended. Each cage card should include the mortality record (number of animals and dates of death).

Random-bred mice are the progeny of matings, within the closed colony, of females and males generally from unrelated litters and different cages. A numerical randomization system based on cage and litter numbers approved by the accrediting agency should be used.

Inbred strains of mice are the progeny of mice resulting from brother × sister matings or from younger parent × offspring matings for twenty or more generations of such matings.

1. A small number of inbred nuclei established at different geographic locations and under the constant supervision of professional mammalian geneticists will supply all breeding stock to accredited commercial producers of inbred mice. There must be close liaison between the supervisor of the specific inbred nucleus and the associated producers supplied by this nucleus.

a. The inbred nucleus shall be propagated only by brother × sister matings or younger parent × offspring matings.

b. Complete records shall be kept for each animal in the inbred nucleus. A standard record card will be recommended by the accrediting agency.

c. To assure the retention of specific genetic and physiological traits by each inbred strain, adequate performance tests shall be conducted at frequent intervals in each inbred nucleus. Performance testing at the final production level will be done if necessary.

2. Production matings.

a. Foundation stock shall be obtained from one of the producers possessing an inbred nucleus.

b. Foundation stock shall be propagated by brother × sister or younger parent × offspring matings.

c. Only one generation of random matings within an inbred strain may be used for production of inbred mice for sale or for production of F_1 hybrids.

F_1 hybrid mice are the progeny from matings of male mice from one inbred strain and female mice from a different inbred strain. Specific F_1 hybrid combinations are useful for different specific laboratory purposes and are not generally interchangeable.

NUTRITION

1. Fresh feed shall be used. It should not be stored longer than 6 weeks after pelleting before use.

2. Feed shall be stored under clean, dry conditions in covered containers.

3. No feed containing unrendered meat scraps (a source of salmonellosis infections) shall be used. The feed manufacturer shall accept and use only rendered meat scraps which have been placed into clean—preferably sterile—containers (e.g., five-ply kraft paper bags) immediately after rendering by the meat packer.

4. It is recommended that no feed be placed in the bottom of cages.

5. Each producer should make sure—especially if he obtains his feed from a local mill—that the feed he uses is:

a. within normal limits of naturally occurring hormone activity and free of diethylstilbestrol contamination;

b. free of additives containing medication or antibiotics;

c. checked for Salmonella.

The results of these assays should be clearly declared by the manufacturer.

6. If an investigator needs mice raised on a particular antibiotic-containing or other special diet, special arrangements shall be made with the mouse producer. This feed, and the mice raised on it, shall be kept in isolation from the regular feed.

The success of the mouse standards in providing the cancer themotherapy programme and other research endeavors with animals nutritionally stable, of known genetic composition and in a healthy state, occasioned the execution of a second contract between the Service Center and the Institute for the period between 15 September, 1957 and 14 March, 1958 for the purpose of preparing standards for commercial rat production. Similar in scope to the mouse guide, the rat standards were published in 1959 (Byerly *et al.*, 1959).

MINIMUM STANDARDS FOR THE COMMERCIAL PRODUCTION OF RANDOM-BRED AND INBRED LABORATORY RATS

FACILITIES AND SANITATION

Facilities, equipment, and sanitary practices shall be designed to provide minimal opportunity for transmission of infectious and parasitic diseases from rats in one cage to rats in other cages, and to rats which may subsequently occupy the same cage. Such practices will minimize the opportunity for the introduction and transmission of infectious and parasitic diseases into and within the producer's premises and from the producer's premises to the user's laboratories.

SANITIZATION: To make physically clean and to remove and/or destroy agencies injurious to the health of laboratory animals.

STERILIZATION: The act or process of killing all living cells, especially microorganisms.

CAGING AND EQUIPMENT:

1. Cages and racks shall be made of a smooth, corrosion-resistant, impervious, easily cleaned and sanitized material, e.g., stainless steel, plastic, or glass, and shall be so constructed as to eliminate or minimize cracks and crevices.

2. Minimum cage area, per nursing female (including litter) shall be:

a. for litters of an average litter size for the colony of one to nine young, 130 sq. in. (when the weaning age is 20-22 days).

Minimum cage area, per rat up to 75 grams, shall be 15 sq. in.

Minimum cage area, per rat 300 grams or more, shall be 50 sq. in.

Cage space for rats of intermediate weights should be adjusted.

3. Water bottles or other watering devices, equivalent in protection of water against contamination, shall be provided.

4. Feed hoppers constructed of corrosion-resistant material shall be suspended in each cage. All hoppers, if contained within the cage, should be covered or should be so constructed as to prevent entry of or gross contamination by rats. They should extend low enough to be reached by pre-weanling rats.

5. Animal room floors, walls, and ceilings should be made of a smooth, hard, impervious material that can be cleaned easily. New buildings should be constructed of materials other than wood.

BEDDING:

1. Bedding shall consist of sawdust, shavings, or other material not readily eaten. Hay, straw, shredded sugar cane, or any other edible material shall not be used.

2. Bedding shall be protected from exposure to vermin prior to use. It should not be obtained from any unprotected storage source.

3. Bedding shall be dry when received and when used.

4. Storage at the rat breeder's establishment shall be under clean, dry, vermin-free conditions.

5. Bedding in mating and holding cages shall be changed weekly or oftener.

BUILDINGS:

1. Animal room temperature should be maintained between 70°F and 80°F. In the summer, buildings can be cooled by painting the roofs with aluminium paint,

attaching sprinkler hose to roof, and using ventilating fans inside the building. Air conditioning is recommended.

2. Adequate, draft-free ventilation shall be provided.

3. Buildings shall be rodent-proof and vermin-free. Dogs, cats, and other pets shall not be permitted in or around the colony.

4. Windows shall be adequately screened when opened for ventilation.

5. It is recommended that the relative humidity of animal rooms be maintained at 40% or above.

CLEANING:

1. Animal house floors shall be cleaned with hot water and detergent once each week. Walls and ceilings shall be cleaned frequently, on a regular schedule, preferably by cleaning with hot water and a detergent having a satisfactory sanitizing efficiency.

2. Corridors and animal room floors shall be swept daily, using a sweeping compound.

3. Cages, feed hoppers, water bottles and watering tubes, or other watering devices shall be very thoroughly cleaned with hot water and detergent or otherwise sanitized before new animals are placed in the cages.

a. Mechanical cage washing machines (operating at 180°F) used with an effective detergent are recommended. If these are used, no separate sanitization of cages is necessary under normal conditions.

b. It is recommended that all mating and holding cages, watering devices, and feed hoppers be sanitized at least once each week.

4. The water bottle or other watering or feeding device removed from any cage shall be returned to the *same* cage unless it is sanitized. This is very important to minimize transmission of diseases.

5. All refuse and dead animals shall be placed in closed containers and removed from the premises daily. Incineration is recommended.

a. There shall be frequent inspection for and removal of sick and moribund animals.

6. Refuse containers and covers shall be sanitized thoroughly after use and before being brought into the animal room.

7. Producers should maintain a rigorous control programme against vermin, rodents, and fly breeding places.

8. Brooms, mops, dust pans, scrapers, and other service equipment shall be kept in a specified storage room.

DISEASE DIAGNOSIS AND PREVENTION

The introduction of new stock to any breeding colony must always be considered a hazard, and every breeder should use every precaution to protect his own stock. Each inbred nucleus colony will practice the strictest sanitary and disease-prevention measures and every effort will be made to deliver the breeding stock in an uncontaminated and disease-free condition.

1. All breeding stock obtained from any source, including the inbred nucleus, shall be quarantined upon receipt for at least 3 weeks in a room or building isolated from the rest of the rat colony.

2. All persons entering the colony shall wash themselves very thoroughly, and dress in special shoes or clean shoe covers, and clean clothing or uniforms before beginning work in the animal rooms. The clothing, uniforms, shoes and shoe covers are to be worn only in the animal rooms and are not to be removed from the animal room until they are removed for laundering. The clothing, uniforms, and shoe covers shall be washed at least once a week or after gross soiling or contamination.

3. Personnel handling animals should wash their hands thoroughly at frequent intervals. Hands should be washed, immediately after handling sick animals, with a disinfectant detergent.

4. Visitors should not be allowed in any animal room.

5. To prevent disease dissemination all rats shall be shipped and delivered in new and non-returnable containers. These containers shall be stored, prior to use, in clean quarters separate from the animal rooms.

6. Periodic examinations of samples of rats, taken from different cages in the colony, shall be made by competent diagnostic laboratories. A minimum of two examinations a year shall be performed at least 4 months apart.

7. Returned animal shipments (for whatever reason) should be considered contaminated and promptly destroyed.

GENETICS AND RECORD KEEPING

Rats distributed by producers shall be identified with respect to ancestry and method of mating used in their production.

Except in instances in which specific genetic or health requirements necessitate the introduction of new stock, maintenance of a closed population with no introduction of outside stock by each producer generally improves the uniformity of the stock and facilitates the maintenance of health in the colony.

The keeping of production records for each mating or breeding pen is recommended. Each cage card should include the mortality record (number of animals and dates of death).

Random-bred rats are the progeny of matings, within the closed colony, of females and males generally from unrelated litters and different cages. A numerical randomization system based on cage and litter numbers should be used.

Inbred strains of rats are the progeny of rats resulting from brother × sister matings or from younger parent × offspring matings for twenty or more generations of such matings.

1. A small number of inbred nuclei established at different geographic locations and under the constant supervision of professional mammalian geneticists will supply all breeding stock to commercial producers of inbred rats. There must be close liaison between the supervisor of the specific inbred nucleus and the associated producers supplied by this nucleus.

a. The inbred nucleus shall be propagated only by brother × sister matings or younger parent × offspring matings.

b. Complete records shall be kept for each animal in the inbred nucleus.

c. To assure the retention of specific genetic and physiological traits by each inbred strain, adequate performance tests shall be conducted at frequent intervals in each inbred nucleus. Performance testing at the final production level will be done if necessary.

2. Production matings.

a. Foundation stock shall be obtained from one of the producers possessing an inbred nucleus.

b. Foundation stock shall be propagated by brother × sister or younger parent × offspring matings.

c. Only one generation of random matings within an inbred strain may be used for production of inbred rats for sale or for production of F_1 hybrids.

F_1 hybrid rats are the progeny from matings of male rats from one inbred strain and female rats from a different inbred strain. Specific F_1 hybrid combinations are useful for different specific laboratory purposes and are not generally interchangeable.

NUTRITION

1. Fresh feed shall be used. Food should not be stored longer than 6 weeks after pelleting before use and a storage time under 4 weeks would be preferable in view of the nutritional loss due to aging.

2. Feed shall be stored under clean, dry conditions in covered, pest-excluding containers, in quarters where temperatures do not exceed 75°F. Nutritional loss is minimized in relatively cool storage places.

3. No feed containing unrendered meat scraps (a source of Salmonella infections) shall be used. The feed manufacturer shall accept and use only rendered meat scraps which have been placed into clean—preferably sterile—containers (e.g., five-ply kraft paper bags) immediately after rendering by the meat packer.

4. Each producer should make sure—especially if he obtains his feed from a local mill—that the feed he uses is:

a. within normal limits of naturally occurring hormone activity and free of diethylstilbestrol contamination;

b. free of additives containing medication or antibiotics.

The results of these assays should be clearly declared by the manufacturer.

5. If an investigator needs rats raised on a particular antibiotic-containing or other special diet, special arrangements shall be made with the rat producer. This feed, and the rats raised on it, shall be kept in isolation from the regular feed.

6. Weaning weight (20-22 days) records and weight curves of males and females up to 12 weeks should be available to users. The weight curves should be re-determined at least annually and these determinations should be based on a random selection of complete litters and a minimum of fifty animals of each sex in each study.

7. The rat undoubtedly is the most widely used animal species for nutrition research. Certain standards of control are of great importance in enhancing the usefulness of this species for this area of research. In particular there is need for standardization and avoidance of change in the formulation of the feed used by any given producer. If a change in feed formulation is of absolute necessity, the producer should be notified immediately so that such information is available to the interested user. Furthermore, the use of "open-formula" and dated feeds is recommended.

During the time the Institute's Committee on Standards was preparing the mouse and rat standards, another Institute panel, the Committee on Animal Husbandry, was establishing a guide for the transportation of research dogs. These were approved for release in December of 1959 and read as follows.

DOG SHIPPING STANDARDS

DIMENSIONS

Dogs transported individually should be transported in crates constructed of a smooth, durable material which is easily cleaned. It should be cleaned before use for each trip. The crate should have a solid floor, which may have a false bottom above it. For four inches above the bottom, the sides of the crate should be solid to prevent the animal's feet from slipping out of the crate. The crate should have openings on two sides and a woven wire top for adequate ventilation. If bedding is used it shall be clean, dry and dust free, and on any one trip it should be cleaned at least every 24 hours and replaced with clean bedding. In all cases the dog should have room enough to be able to stand erect in the crate, and room enough to lie down in an extended position. Dogs transported in individual crates should occupy crates of at least the following dimensions.

For a dog up to 25 lb	16 in. by 24 in. by 18 in.;
for a dog up to 35 lb	18 in. by 30 in. by 22 in.;
for a dog up to 45 lb	20 in. by 36 in. by 28 in.;
for a dog up to 60 lb	24 in. by 40 in. by 32 in.;
for a dog over 60 lb	24 in. by 46 in. by 36 in.

Groups of dogs transported in pens should be provided with the following minimum space assignments.

For dogs up to 25 lb	2 sq. ft per dog, 28 in. high;
for dogs up to 35 lb	3 sq. ft per dog, 30 in. high;
for dogs up to 45 lb	4 sq. ft per dog, 32 in. high;
for dogs over 45 lb	5 sq. ft per dog, 32 in. high.

In hot weather (over 85°F) the spacing per dog should be increased by 10%.

FOOD AND WATER

Dogs which will be in transit for 4 hours or more should be offered food 2 hours before loading and fresh water about 30 minutes before loading. Food and water should not be offered immediately before loading. On long trips dogs should be offered food at least once for each 24 hours. All dogs should be offered water at least at 12-hour intervals, and when the temperature reaches 90°F all dogs should be offered water at 4-hour intervals. Newly weaned pups should be offered food at 4-hour intervals with food designed for pups. Food and water containers should be cleaned and sanitized before each trip. Disposable paper containers may be used and disposed of after being used once.

THE TRUCK

Dogs should have protection from the sun in hot weather and protection from cold weather. There should be arrangements for ventilation, especially in hot weather, and arrangements for making the truck draft-free in cold weather. Arrangements should be made for warming an area carrying weaned pups if the temperature falls below 50°F and for warming an area carrying unweaned pups if the temperature falls below 65°F.

If the truck has a closed body, the body and motor exhaust system should be designed and maintained so that no *significant* amounts of carbon monoxide can gain entrance to the cargo space. Such a truck should have a system for forced ventilation if the animals are to be transported when the temperature exceeds 90°F.

GENERAL

Only dogs that are compatible should share the same pen. No bitch obviously about to whelp should be transported. No bitch in season should be placed in a pen with male dogs. Each dog should be inspected at 4-hour intervals, or oftener.

Only healthy dogs should be shipped and they should meet the health requirements of the State into which they are going.

The same Committee, recognizing the necessity of providing laboratory animal users with a series of acceptable standards for the utilization of biological material, established two sets of principles for workers at the university-industrial level and at the high school-science fair level. The first, for professional personnel, reads.

PRINCIPLES OF LABORATORY ANIMAL CARE

1. All animals used for experimental purposes must be lawfully acquired and their retention shall be in strict compliance with Federal, State, and local laws and regulations.
2. Research projects involving live animals must be performed by, or under the immediate supervision of, a qualified biological scientist.
3. The housing, care, and feeding of all experimental animals shall be supervised by a properly qualified veterinarian or other biological scientist competent in such matters.
4. All laboratory animals must receive every consideration for their comfort; they must be kindly treated, properly fed, and their surroundings kept in a sanitary condition.
5. Rooms in which animals are to be housed shall be provided with a floor which can be kept clean, and the room shall be lighted and ventilated. The temperature shall be held within reasonable limits. Cages should be of sufficient size to permit the animals used to stand or lie in a normal position. It is generally conceded that animals maintained for long periods are in better physiological condition if they exercise regularly. Species housed out of doors should be given adequate protection from direct sunlight or inclement weather.
6. The food and water supplied to all experimental animals, subject to the nature of the research, must be palatable, and of sufficient quantity and proper quality to maintain the animals in good health.
7. In any operation likely to cause greater discomfort than that attending anesthetization, the animal shall first be rendered incapable of perceiving pain and be maintained in that condition until the operation is ended.

a. Whenever anesthetization would defeat the purpose of the experiment then the experiment must be approved by the head of the department and directly supervised by the head of the laboratory.

b. If an acute study does not require survival, the animal must be killed in a humane manner at the conclusion of the experiment.

c. If the nature of the study is such as to require survival of the animal, then acceptable techniques must be followed throughout the operation.

8. The post operative care of animals must be such as to minimize discomfort during convalescence in accordance with acceptable hospital practice.

9. The care and housing of individual species should be in accordance with the recommendations of the Institute of Laboratory Animal Resources (NAS-NRC) as these are issued or revised.

The second, for students, is as follows.

GUIDING PRINCIPLES IN THE USE OF ANIMALS BY SECONDARY SCHOOL STUDENTS AND SCIENCE CLUB MEMBERS

The basic aim of scientific studies that involve animals is to achieve an understanding of life, and to advance our knowledge of the processes of life. Such studies lead to a respect for life.

Insects, other invertebrates and protozoa are materials of choice for most experiments. They offer opportunities for exploration for biological principles and extension of established ones. Their wide variety and the feasibility of larger numbers than is usually possible with vertebrates makes them especially useful for illustrating principles.

1. A qualified adult supervisor must assume primary responsibility for the purposes and conditions of any experiment that involves living animals.

2. No experiment should be undertaken that involves anesthetic drugs, surgical procedures, pathogenic organisms, toxicological products, carcinogens, or radiation unless a biologist, physician, dentist, or veterinarian trained in the experimental procedure involved assumes direct responsibility for the proper conduct of the experiment.

3. Any experiment must be performed with the animal under appropriate anesthesia if the pain involved is greater than that attending anesthetization.

4. The comfort of the animal used in any study shall be a prime concern of the student investigator. Gentle handling, proper feeding, and provision of appropriate sanitary quarters shall be strictly observed at all times. Any experiment in nutritional deficiency may proceed only to the point where symptoms of the deficiency, appear. Appropriate measures shall then be taken to correct the deficiency or the animal killed by humane methods.

5. All animals used must be lawfully acquired in accordance with state and local laws.

6. Experimental animals should not be carried over school vacation periods unless adequate housing is provided and a qualified caretaker is assigned specific duties of care and feeding.

The demonstration of the value of standards in the production of commercially available rats and mice led the Institute in 1960 to

decide upon a more comprehensive approach to the provision of standards for research animal production, care, maintenance and utilization. The first fruits of this approach was the acceptance, by the Institute Governing Board of standards for hamsters. Prepared by the Committee on Hamster Standards, chaired by Dr. T. C. Byerly, this guide was approved in December 1960.

STANDARDS FOR THE BREEDING, CARE, AND MANAGEMENT OF SYRIAN HAMSTERS

INTRODUCTION

The Syrian hamster, *Mesocricetus auratus*, has had a relatively short history as an experimental animal. Descendants of the original groups that were captured in Syria in 1930 were subsequently brought to England, and still later to the United States. This species is being used extensively in infectious and parasitic disease investigation, vascular studies, nutrition research, dental research, the study of compounds that demonstrate estrogenic activity, and studies that involve observations concerning tumors.

In research, it is of great importance that the animal material used be as uniform and standardized as possible, in order to obtain repeatable results within a laboratory, or between laboratories. Uniformity of biological material, likewise, increases the accuracy of results, and improves the efficiency of research by decreasing the number of repetitive experiments required to ascertain that a particular result is not due to chance.

As with other laboratory animals, genetic and environmental factors, acting separately or concurrently, contribute to biological variability in the hamster. Thus, to increase uniformity, controls must be applied to both. The control of genetic variability can be accomplished to a considerable extent by the rigid application of a particular mating system(s) and selection pressures, depending on how much uniformity is required and the particular characteristic(s) involved. The important fact to keep in mind, is that genetic uniformity is possible (even though individual variation within a population is evident), if the mating system and selection criteria initially established are consistently followed. The control of environmental factors, likewise, can be accomplished to a considerable extent by the establishment of, and adherence to, uniform and optimal conditions and procedures in colony management. Strict controls of cleanliness, nutrition, temperature and humidity, and disease prevention, for example, will promote the development of environmental uniformity, thus contributing to the over-all uniformity of the animals.

Though both genetic and environmental uniformity are difficult to obtain, the judicious application of current knowledge and experience will permit much progress to be made. The standards herein presented are the result of this knowledge and experience.

The Committee on Standards realizes that additional information on housing, nutrition, genetics, husbandry practices, diseases, and other problems is urgently needed. It is hoped and urged that as more knowledge and experience is obtained, the Committee will be so informed, so that such new material can be incorporated in future revisions.

The Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council, strongly urges these standards be adopted by producers and users alike. Past experiences with the *Minimum Standards for the Commercial Production of Random-bred and Inbred Laboratory Mice* has given unquestionable evidence of the extreme value of such standards. The marked improvement of physical facilities, husbandry practices, genetic procedures, and other aspects of laboratory animal care and production, as well as a reorientation of concepts and philosophies during the past three years has been most profound. As a result, strong support from the laboratory animal industry, governmental agencies, the pharmaceutical industry, and from academic groups has been clearly evident. Standards previously prepared by the ILAR have been voluntarily adopted by producers as their guiding principle, and by industrial concerns and governmental agencies as their purchasing specifications. It is quite likely the standards for hamsters will be similarly adopted.

GENERAL CONSIDERATIONS

1.00 GENERAL: The following standards are based on present-day knowledge and experience in the care and maintenance of the Syrian hamster, *Mesocricetus auratus*. These are to be applied to colonies maintained for experimental purposes as well as for production colonies.

1.20 FACILITIES, EQUIPMENT, AND OPERATION: The facilities, equipment, and husbandry procedures shall be designed and operated to afford maximum environmental control, optimal comfort and welfare for the animals, and minimal opportunity for the transmission of diseases and parasites from one animal to another, and from group to group.

1.21 The physical facilities shall be designed and constructed so that clean and soiled material and equipment will be maintained separately, and shall afford maximum control of temperature, humidity, and light, so that the animals shall have optimal conditions for their comfort and welfare.

1.22 The caging equipment and feeding and watering devices used shall be designed and fabricated so as to afford maximum comfort and welfare for the animals, minimum opportunity for the transmission of diseases and parasites, and ease and efficiency of sanitizing and sterilizing.

1.23 Auxiliary equipment such as washing machines, cage racks, rolling equipment (dollies, tables, carts, etc.), and fixed equipment (cabinets, sinks, etc.), shall be designed and fabricated according to the best available knowledge, and shall be used in such manner as to promote maximum environmental control and efficiency in operation.

1.24 Operating procedures shall be performed according to the recommendations of these standards, and shall consist of those practices that will include the best information and experience in nutrition, genetics and animal breeding, care and maintenance, colony management, disease control, etc., so that maximum environmental control is accomplished, and optimal conditions for the comfort and welfare of the animals are provided.

1.30 DEFINITIONS: For the purposes of these standards, sanitization and sterilization are defined as follows:

SANITIZATION: To make physically clean and to remove or destroy agents injurious to the health of laboratory animals.

STERILIZATION: The act or process of killing all living cells, especially microorganisms.

FACILITIES—CONSTRUCTION

2.00 EXTERIOR WALLS shall be of masonry or metal. Wooden construction other than studding, plates, laths, etc., will not be acceptable. The outside and inside surfaces of these walls shall be rendered impervious to liquids and moisture, rodent and vermin-proof.

2.01 The inside surfaces may be covered with coatings or sheeting, and shall be smooth and hard without pitting and/or cracking, and shall be easily cleaned.

2.02 All welded, caulked and mortared joints shall be completely filled.

2.10 INTERIOR WALLS (interior bearing walls and partitions) may be of masonry, metal, masonite, fibreboard, flexboard, transite, dry wall, cement plaster, or comparable material, provided that the finished surfaces comply with the specifications noted above for the inside surfaces of the exterior bearing walls. Wooden studding is acceptable for interior walls and partitions.

2.20 ROOFS may be constructed of any of the materials that are commonly used for this purpose by the building trades.

2.21 Roof trusses, ridge pole (ridge piece, ridge plate), wall plates, tie beams, king posts, struts, ple plates, and rafters may be of wood.

2.30 CEILINGS shall be used, and constructed of materials equal to those suggested for walls and partitions, and shall be subject to identical finishing conditions.

2.31 Access panels in ceilings shall completely fill the opening when in a closed position.

2.40 NEW FLOORS shall be constructed of dense mix concrete or equivalent with a smooth surface, coated with a sealer to render them impervious to liquids and moisture, to prevent dusting and for easy cleaning. A waterproof membrane is recommended.

2.41 Floors constructed of wood may be used provided that they are completely covered with metal, linoleum, tile, etc.

2.42 If metal is used, it shall be corrosion resistant and fastened to the wood without buckling or warping. It shall present a smooth and unbroken surface, and all joints, including those at the walls, shall be closed.

2.43 If linoleum or tile is used, it shall be firmly affixed to the floor with a suitable moisture and liquid resistant adhesive. The upper finished surface shall be smooth and unbroken, and all joints shall be completely filled.

2.50 DOORS shall be provided for all rooms. These doors shall be so located as to allow entrance and exit to clean and dirty areas. There shall be no intercommunicating doors between rooms.

2.51 Each door shall have an adequate door latch and lock, and shall be rodent and vermin proof when in closed position.

2.52 Door frames shall be sealed to the walls and partitions with caulking or similar material.

2.60 OUTSIDE WINDOWS are not desirable. It is suggested that a viewing port of transparent glass be installed for inspection purposes.

2.70 LIGHTS shall be of a type and in a location that simplifies cleaning. If ultraviolet lights are used, they should be of a design that limits the production of ozone below toxic levels (Mittler *et al.*, 1957; Mittler and Ehrlich, 1958).

FACILITIES—AIR CONDITIONING

3.00 AIR CONDITIONING in animal quarters is required. Recirculated air can be used if the system is provided with equipment that can remove and/or destroy all microorganisms. Recirculation is not recommended.

3.01 Animal-room temperatures shall be maintained between 69° and 75°F. It is recommended that 69° to 71° be used for adults and young hamsters in holding rooms, and 72° to 74°F for breeding rooms.

3.02 Relative humidity levels should be maintained between 40% and 60%.

3.03 Each room shall be provided with air conditioning and humidity controls. It is recommended that graphic recorders be used for tabulations of 24-hour system performance.

3.10 DUCT WORK, preferably, should be located above the ceiling or flush mounted. However, if installed below the ceiling, the upper surface of the ductwork shall be sealed to the ceiling or suspended at least 6 in below the ceiling to facilitate cleaning.

3.11 The diffusers and exhaust openings shall be so located and controlled as to avoid drafts.

3.12 Openings in ventilation grillwork shall not be of a size that will permit the entrance of rodents.

3.20 THE SUPPLY of air shall be such as to provide a minimum of six air changes per hour.

3.21 The air pressure within clean spaces shall be greater than that in public and refuse areas.

3.30 CIRCULATING FANS may be used during periods of air conditioning system breakdown, repairs, etc.

3.31 Supplemental exhaust fans, if used, shall be permanently mounted in external window or wall openings and screened. Their frame shall be sealed to the building structure.

3.40 STANDBY GENERATORS should be available in the event of power failure.

3.50 CENTRAL HEATING, if used, should be compatible with the requirements stated above.

EQUIPMENT

4.00 CAGES shall be fabricated of a smooth, corrosion-resistant material. It shall be impervious to liquids and moisture, easily sanitized and/or sterilized. Materials that are considered acceptable include plastics, galvanized metal, stainless steel or other stainless metal alloys, glass, aluminum (hard alloys), and magnesium. Wood is not acceptable. The painting of cages and racks is not recommended.

4.01 All cages shall have water-tight seams. Fabrication methods used shall be performed in a manner that shall not create crevices.

4.02 All cages shall have a lid (cover) to prevent the escape of caged animals and the entrance of stray animals.

4.03 The minimum cage area for a nursing female, including a litter of any size, shall be 150 sq in, and the minimum depth of the cage shall be not less than 6 in. The following table lists the allowable floor space per hamster according to age groupings:

Age	*Sq. In. per Animal*
Weaning to 5 weeks	10·0 or more
5 weeks to 3 months	12·5 or more
Over 3 months	15·0 or more

4.10 RACKS (stands) shall be fabricated of a smooth, corrosion resistant material. It shall be impervious to liquids and moisture, easily cleaned, sanitized and sterilized.

4.11 If racks are fabricated of pipe or tubular material, all openings shall be closed without crevices.

4.12 Racks may be of fixed or portable design, arranged to facilitate cleaning activities. Wood is not acceptable.

4.20 FOOD HOPPERS, *if used*, shall be constructed of any durable material other than wood, shall be resistant to the gnawing of rodents, and corrosion resistant when exposed to acids, alkalis, detergents, moisture, liquids, and excreta. They shall be easily sanitized and/or sterilized.

4.21 The food hopper shall be suspended in a manner that allows at least one inch of clear space between the lower surface of the food hopper and the upper surface of the bedding. The food hopper may be hung from the edge of the cage, or from the cage lid, or formed as part of the cage lid, or inserted through and supported by the cage lid.

4.30 WATER BOTTLES shall be used; they should be formed of clear glass, and preferably not over ½ pint in capacity.

4.31 They shall be mounted or suspended in a manner that will prohibit contact by the caged animals.

4.32 If drinking tubes are used, the tube shall be of corrosion resistant and durable material. Aluminium, copper, and alloys containing copper are not recommended.

4.33 Perforated bottle caps that are used in lieu of a combination of stopper and tube, are acceptable.

4.40 MECHANICAL CAGE WASHER should be used by producers and users.

4.41 It should be of a design that will insure a continuous supply of hot water at 180°F for all phases of the washing and rinsing cycle. The duration of the complete cycle shall not be less than 3 minutes.

4.42 There should be at least one rinse, after the articles have been washed with a detergent solution. If only one rinse is used, it shall be supplied directly from the fresh hot water supply line.

4.43 The washing machine shall be equipped with pump pressure gauges and a thermometer for each phase of the cycle.

4.44 It is recommended that the washing machine be equipped with an automatic detergent dispenser and a temperature control.

4.50 OTHER EQUIPMENT. Each room shall have a small sink, primarily for the washing of hands. It should not be used for any other washing purpose.

4.51 The sink shall be supplied with hot and cold water; dispensers for soap, detergent, and bactericide, separately or as combinations; and a paper towel dispenser.

4.52 Cabinets, work benches, and carts, *if required*, shall be so located as to facilitate cleaning. It is recommended that these pieces of equipment be portable.

4.60 STORAGE. Equipment and material not in constant use, or which will not be required in the immediate future, shall not be stored in any animal room.

FOOD AND BEDDING

5.00 GENERAL: Food and bedding are two items of expendable material that are purchased by producers and users. Both are generally used in animal facilities without prior treatment, and hence are potential sources of contamination for diseases, parasites, and abnormal hormonal stimulation. It is, therefore, urged that producers and users be aware of these problems and exercise care in their purchase and storage.

5.10 FOOD. The production colony management should obtain the vendor's assurance, and if possible receive guarantees, that the pelleted feed that he uses is:

a. Within normal acceptable limits of naturally occurring hormone activity.

b. Free of additives containing drugs, hormones, or antibiotics.

c. Examined for the presence of *Salmonella.*

d. Free from rodent and vermin contaminations.

5.11 Feed shall not be accepted by the production colony management unless it is delivered in clean and sealed containers made of new material.

5.12 Feed received shall not be accepted unless it is marked with the milling date. Feed older than 6 weeks from date of milling shall not be fed to the animals. The milling date should be plainly printed on the bag, preferably not in code.

5.13 Feed shall be stored in a clean, dry, rodent and vermin-free area in covered containers with tightly fitting lids, or in their original sealed containers.

5.14 Pelleted food shall be supplied, perferably, on a daily basis. Under no circumstances, shall any cage be provided with more than one week's supply of these prepared foods except during the early lactational period.

5.15 Pelleted food that is present in food hoppers or cages when cages are scheduled for washing, shall be discarded.

5.16 Feed supplements, in the form of kale, carrots, apples, etc., if used, shall be washed well.

5.17 This food should be offered in limited quantities and may be placed on the bedding for immediate consumption. Supplemental food that is left over from the previous day's feeding shall be removed daily.

5.20 BEDDING. It is recommended that bedding shall be of a composition that is not readily eaten by the animals. It shall not contain substances which are injurious when ingested. Resinous and hardwoods are not recommended. White pine coarse sawdust and shavings are recommended. Cedar, basswood, poplar and crushed corn cobs are acceptable.

5.21 Bedding should not be purchased from a source whose storage facilities are not adequately protected from vermin and rodent contamination.

5.22 Bedding shall be obtained from the vendor in a container which is non-returnable. If obtained in bags, they shall be non-porous and sealed (i.e., burlap bags shall not be used). Baled bedding shall be obtained in closed containers, i.e., paper or plastic bags, paper wrappings, etc.

5.23 The bedding shall not contain more than 12-15% moisture when received.

5.24 The bedding shall be stored in dry, rodent and vermin-proof, frequently sanitized containers or storage areas.

COLONY OPERATIONS

6.00 GENERAL. Procedures in the operation of a colony are of utmost importance, for they determine the extent of the environmental control exercised. The latest design in physical facilities and equipment are no better than the procedures used in operating them. It is, therefore, strongly recommended that producers and users alike constantly review (and revise) their operating procedures, so that optimal conditions of care and management are provided for the animals.

6.10 CLEANING (FLOORS, WALLS, CEILING, VIEWING PORTS, ETC.). Floors shall be swept and cages and racks dusted at least once each day. The completion of the day's activities should include a complete dusting and sweeping.

6.11 The use of counter and radiator brushes is acceptable for dusting activities; however, moistened sponges are preferred.

6.12 Equipment on casters shall be moved for sweeping purposes. Pushbrooms are preferred for they do not create as much dust as straw brooms. The use of sweeping compounds or moistened sawdust is recommended.

6.13 Floors shall be washed at least once each week with a solution containing a detergent and a bactericide.

6.14 Walls, ceilings, view ports, doors, etc., shall be washed at least once every 3 months with a solution containing a detergent and a bactericide.

6.15 Equipment other than animal cages and racks shall be dusted daily and washed with a solution containing a detergent and a bactericide at least once every 3 months.

6.20 CLEANING (CAGES, RACKS, FEEDERS, BOTTLES, ETC.). Soiled bedding shall be removed and replenished in the cages at least twice per week. The water supply in each bottle shall be replenished as necessary, preferably on a daily basis. Left over water shall be removed from the bottle prior to refilling. If bottles are not washed, they shall be returned to the cage from which they were removed. It is recommended that water bottles be filled by an automatic bottle filler, or by means of a hose, mounted on a spring loaded reel, and a self-closing faucet. The nozzle of the faucet shall be so designed that it cannot be inserted into the bottle.

6.21 Animal cages and racks shall be washed at least once every 2 weeks. At least once per week is recommended.

6.22 Water bottles, bottle stoppers, drinking tubes and food hoppers should be washed at least once each week in an effective cage washing machine. It is recommended that water bottles, stoppers and tubes be washed daily.

6.30 REMOVAL OF REFUSE. All refuse and cage cleanings shall be placed in metal or plastic containers with closely fitting lids or whose tops can be fastened securely. Acceptable kinds of containers include metal cans, plastic cans, plastic or five-ply paper bags, non-reusable cartons, etc. Uncovered tubs, burlap sacks, baskets, etc., are not acceptable for this purpose.

6.31 Reusable containers for refuse shall be sanitized after they have been emptied. Partially filled containers shall not be held over until the following day.

6.32 Refuse containers that are positioned outside of the building and are filled via a chute, shall be sanitized after they have been emptied, at the end of the working day.

6.33 Refuse chutes shall be sanitized at the end of the working day.

6.34 The incineration of dead animals, refuse, soiled bedding, and used disposable containers is strongly recommended.

6.40 DISEASE AND PARASITE CONTROL. All new breeding stock and new acquisitions shall be quarantined for a period of at least 2 weeks in a separate building, or in a special room that is not associated with a production colony. A holding room or an animal experimental room is not acceptable. During this period suitable laboratory observations shall be made. It is recommended that newly acquired breeding stock be quarantined and observed through the second filial generation. These observations should include necropsies of all members of preceding generations, especially the retired breeding animals.

6.41 Personnel assigned to quarantine areas should not work in other animal rooms, and vice versa. If the above arrangement is not practical, it is recommended that the quarantine areas be serviced after all chores have been completed in the other animal rooms.

6.42 All persons entering the animal area should shower, in any case wash themselves thoroughly, and dress in clean outer clothing and special shoes or shoe covers. The clothing and shoes or shoe covers shall be worn only in the assigned work area, and shall not be removed from this area except for cleaning. Sanitization of these articles shall be performed at least once each week.

6.43 Dead animals shall be removed immediately as detected. They shall be placed in containers as specified in Sec. 6.30. All sick animals (other than those concerned with research) shall be removed when detected, and sacrificed immediately in a humane manner. It is recommended that the daily caretaking routines be so arranged that the animals will be observed at least twice daily. Wherever possible, all dead animals and all the sick which have been sacrificed should be examined daily and necropsies performed. Necessary diagnostic procedures should then be carried out by trained personnel.

6.44 Hands shall be washed with soap and a bactericide after sick and dead animals have been handled, even though forceps or other devices are used in the process. If non-disposable devices are used for this process, they shall be sanitized after use in one cage, and before they are used in another.

6.45 Periodic examinations of samples of hamsters, taken from a representative sample of the cages in the entire production colony (including holding rooms) shall be made by a competent diagnostic laboratory. These samples shall be submitted once each month, and the examinations will include tests for the presence of *Salmonella*, enteritis ("wet tail"), and endo- and ecto-parasites.

6.46 An effective vermin and rodent control program shall be maintained.

6.47 Pets shall not be allowed in or near the animal rooms.

6.48 All hamsters shall be shipped from colonies in non-returnable containers. These containers shall be made of new material, and shall be assembled, stored and filled in clean quarters that are separate from the animal rooms. Hamsters shall not be returned to a production colony once they have been removed.

6.49 Undeclared antibiotics and other drugs may not be used in a production colony.

6.50 VISITORS. Visitors shall not be allowed in any animal room, quarantine area, nor in any space that is considered to be a clean area, unless they wash themselves thoroughly and don approved coverings (hair, body and feet) before entering an animal room. The number of visitors should be severely restricted.

6.60 RECORD KEEPING. Proper records shall be kept by the production colony

management in order to (1) determine the efficiency of the operation, (2) trace the origin and spread of diseases, and (3) determine biological performance. It is recommended that users of hamsters maintain mortality records and other pertinent data for animals in holding and quarantine rooms; such data will be of value in determining the efficacy of quarantine procedures, and will permit them to relate their animals to the originating colony.

6.61 Producers shall maintain records for each cage. The following record forms are recommended.

1—Record of Breeding Females

Date of Birth of ♀ ♀ *No.*..... *Parents: Father*.....
Mother.....

Mated to ♂ No.	Litter No.	Date Mated	Date Litter Born	Date Litter Weaned	No. Young Born	No. Young Died	No. Young Weaned	♂♂ Weaned	♀♀ Weaned	Unproductive Matings	Remarks

2—Record of Breeding Males

Date of Birth of ♂ ♂ *No.*..... *Parents: Father*.....
Mother.....

Mated to ♀ No.	Date Mated	No. Young Born	No. Young Weaned	Unproductive Matings	Remarks

3—Summary Sheet

Date	No. Young Died	No. Litters Eaten	Dead Mothers	Dead Litters	Dead Mothers and Litters	Litters Destroyed	Litters Weaned	Young Weaned	Litter Average

Tables 1 and 2 are individual record cards. The remarks column can be used to indicate the final disposition of the breeding animals. Table 3 is a summary sheet that can be assembled on a daily, weekly, monthly, seasonal and annual basis.

6.62 Adequate records of performance tests conducted at frequent intervals on a representative sample of the breeding colony shall be maintained, to assure the retention of desired traits.

6.63 Weaning weight records and weight curves of males and females up to 12 weeks of age shall be made available to users by producers. The weight curves shall be re-determined at least once annually, and these determinations shall be based upon the random selection of complete litters of various sizes, with a minimum of fifty animals of each sex in each determination.

6.70 BREEDING AND MATING SYSTEMS. The hamsters shall be bred in accordance with an approved mating system.

Pursuant to the 1960 decision noted above, the Institute and its Committees are committed to a long-range programme for the development and establishment of standards for the production, procurement, care, maintenance, shipping and use of the common species of experimental animals. Presently, the Committees on Primates and Laboratory Animal Transportation are engaged in the preparation of standards for the shipping of research primates and rodents, respectively. Other Institute committees, to be appointed in the near future, will study the need for revision of the existing mouse and rat guides, and will establish production standards for the guinea pig and the rabbit. At a later date, similar standards will be erected for dogs and cats.

It should be emphasized that the enforcement of standards established by the Institute is, and will be in the future, purely a voluntary act. The Institute of Laboratory Animal Resources, an agency of the National Academy of Sciences-National Research Council, is a private, non-profit organization, which under the limits of its establishing Charter received from the Congress of the United States, does not engage in licensing or enforcement activities. Nevertheless, voluntary implementation of existing standards is being effected through the incorporation of these standards in animal bid specifications and purchase contracts.

REFERENCES

Byerly, T. C. *et al.* (1957). *J. nat. Cancer Inst.* 19, 1137-1143. Minimum Standards for the Commercial Production of Random-Bred and Inbred Laboratory Mice.

Byerly, T. C. *et al.* (1959). *J. nat. Cancer Inst.* **22,** 869-876. Minimum Standards for the Commercial Production of Random-Bred and Inbred Laboratory Rats.

Mittler, S. and Ehrlich, R. (1958). *J. infect. Dis.* **103,** 145-149. Susceptibility to Respiratory Infections of Animals Exposed to Ozone. I: Susceptibility to *Klebsiella pneumoniae.*

Mittler, S., King, M. and Burkhardt, B. (1957). *A. M. A. Archives of Industrial Health* **15,** 191-197.

2. THE BRITISH ACCREDITATION SCHEME

A note on the commercial breeding of small animals for laboratory use

The breeding of animals for research and diagnosis requires an initial outlay of capital on buildings, hutches, cages, etc., access to feeding-stuffs and other materials, and a lot of knowledge which is not by any means all to be found in books. Moreover, profits are not large and are never likely to be, although the successful breeder should find it worth his while. Capital outlay must be severely restricted if it is not to neutralize eventual profits, and full advantage must be taken of existing facilities. Running costs have to be watched continually, whether feed is grown or bought, and the proper balance must be struck between false economy and extravagance unrewarded by improved output. When it comes to management, knowledge and experience count for most of it, and neither is easily come by.

In giving an outline of the Laboratory Animals Centre's Accreditation Scheme for breeders of guinea-pigs, rabbits and mice, it is necessary to review briefly the circumstances which led to its formation in 1950.

Laboratories found in the past that the stock they were purchasing from commercial breeders had very often been infected and important experiments had to be interrupted or abandoned because of outbreaks of disease due to infection in the stock. The great variation in animals offered to laboratories also caused dissatisfaction as for some types of experiment an animal conforming to definite standards is absolutely necessary. The scheme was introduced to help serious breeders produce the right sort of animal.

Breeders who are interested in becoming accredited are in the first place asked to complete a questionnaire. If the answers given on this questionnaire indicate that the breeder is likely to satisfy the conditions for accreditation, arrangements are made by the Centre for a visit to the breeder's premises by a suitably qualified person. If the inspection of the premises and any called for laboratory reports are satisfactory, the breeder receives a *Certificate of Accreditation* from the Centre, valid for six months. It is usually renewed without further inspection or laboratory examination, provided there is no reason to think the quality of the animals has fallen below accreditation standards.

The requirements for accreditation are as follows:

(*a*) Before being considered for accreditation the breeder should be breeding, or intend to breed, not less than 400 guinea-pigs, 200 rabbits or 5,000 mice a year. (In recent years these minimum numbers have been increased considerably.)

(*b*) The stock must be raised predominantly, if not entirely, for laboratory use.

(*c*) The colony must be strictly self-contained; if it is desired to bring in stock from outside, the Laboratory Animals Centre must be consulted beforehand.

(*d*) The breeder must supply only animals of his own breeding to the laboratory, and not those from any other source. Buying-in for re-sale is absolutely prohibited.

(*e*) The standards of housing, caging, feeding and general management and hygiene shall be compatible with the production of first-class animals.

Some advantages which accrue from the accreditation scheme are listed below.

(*a*) Arrangenents have been made whereby the breeder can have a free laboratory examination of animals which may die, in order to establish the cause of death. Special arrangements, over and above this, apply to accredited guinea-pig breeders.

(*b*) The Centre publishes a register of accredited breeders to all laboratories where animals are used experimentally (as well as to all accredited breeders). A twice monthly *Parade State* also goes to laboratories showing what animals are currently available. The breeder thus stands a better chance of making a contract with a laboratory.

(*c*) Although it does what it can to remove any conflict between the interests of breeder and user, the Centre can offer no guarantee that accredited breeders will find a market for their animals. It does, however, encourage the laboratories, in their own interests, to give preference to accredited animals.

Further information, and application forms for accreditation, may be obtained from the Laboratory Animals Centre, M.R.C. Laboratories, Woodmansterne Road, Carshalton, Surrey.

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