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Animals for Research

Principles of Breeding and Management

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Principles of Breeding and Management

Edited by W. Lane-Petter

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

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Preface

Laboratory animal science is a comparatively new subject. There are some who will protest that its designation is premature and that it has not yet emerged from an ill-favoured technology. Certainly the literature in this field is still so modest that the student has some possibility of making himself familiar with the greater part of it. But this primitive stage is not destined to last; indeed, the end of it is already in sight, and the present book is contributing towards that end.

A few other compendiums or comprehensive reference books on laboratory animals have been published, and have received their measure of acclaim. It was thought, however, that there was a need for a book that concentrated on the species most commonly used, and that dealt with them more profoundly than has elsewhere been customary. This book is written mainly for users of such animals, but also for those who have charge of what have been called user-breeder colonies. Those who want information about the more out of the way species must seek elsewhere, paying the penalty for being in the minority; here only the majority will be served. More than that, special emphasis in this book has been given to mice and rats, whose numerical preponderance over all other vertebrate species, and whose pre-eminent usefulness in the laboratory, place them almost in a separate category. Of all experimental animals they begin to approach the ideal of an exact reagent or an accurately definable and reproducible system, and it is for that reason that nearly half this book is concerned with them. Even the general chapters have mice and rats chiefly in mind.

No apology is offered for this lop-sidedness, for it is deliberate, and it is hoped that it will meet the needs of many research workers. There are, however, chapters specifically dealing with the second echelon of animals, whose use is less numerous or ubiquitous but still common. The treatment is not so profound as in the case of mice and rats, but it is nowhere superficial.

The editor is grateful to all those who have contributed chapters to this book, and counts himself as greatly privileged to have had their collaboration. Their knowledge, and in so many cases their original approach, have made the construction of the book an exciting as well as an exacting labour. Thanks are due also to the many colleagues who have helped with criticism and suggestion, and in more tedious ways, such as proof reading. All of us must hope that this book will help towards better research, in so far as research relies on better managed animals used in the most profitable ways. An experimental animal—and this applies above all to highly defined strains and types of mice

and rats—is part instrument, part reagent, a complicated and incidentally sentient system. To get the maximum information out of it, by calibrating and using it to the best advantage, is sensible economy as well as profitable research. An understanding of the nature, capabilities and limitations of the animal is also likely to lead to its humane use; and end which may be incidental but is always desirable.

W. LANE-PETTER

Carshalton, 1963

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Chapter 1

The Physical Environment of Rats and Mice

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I. INTRODUCTION

Some years ago, in the course of the discussion following a lecture on the breeding of laboratory animals, a lady ended an impassioned speech of protest with the rhetorical question "How would you like to spend all your life within the confines of a little wire cage?" The lecturer, taking the question quite literally, replied that he would most certainly object but that unlike laboratory rats and mice he was not the product of countless generations of laboratory breeding.

Nevertheless, the lady had a point. Animals, including laboratory rats and mice, are complicated biological systems. Their lives are a constant round of activity—feeding, mating, reproducing and interpersonal relationships—which is governed by an innate pattern of behaviour characteristic of each species and, within limits, varying between different populations and even between individuals. Their environment must allow them to realize the innate potential activity that constitutes their lives, or at least such aspects of it as are desirable from their consideration as laboratory animals. Failure to take account of this must result in reducing the efficiency of the system; in retarded or abnormal growth, poor reproduction, incompetent mothering leading to increased infantile mortality, fighting and other anti-social behaviour, impaired health, cannibalism, viciousness and so on. Put in more subjective terms, if you want your rats and mice to grow and reproduce to the best of their ability, you must provide them with quarters that are comfortable and adequate.

II. The Wild Archetype

All laboratory species have been derived from wild ancestors. In the course of breeding in captivity they have been altered, often profoundly, but their derivation is still completely self-evident. Laboratory rats and mice, of whatever unusual strains, are still immediately recognizable for what they are; much more so, perhaps, than many breeds of dog. It is reasonable to postulate that their innate pattern of behaviour may also be to a very large extent archetypal.

There are, of course, obvious differences, both in the physical appearance and in behaviour, between the wild and the laboratory bred versions. Colonies of albino rats, or of mice with a high incidence of leukaemia, for example, do not occur in nature. Similarly, the laboratory versions are normally tame and easily handled, while the wild ones can be guaranteed to bite if cornered. If it were possible to feed laboratory rats on exactly the same diet as wild rats subsist on in nature, it is doubtful whether the former would thrive.

In the course of laboratory breeding the group behaviour of rats and mice has undergone profound modification of the archetypal pattern. On the other hand, laboratory rats and mice which are normally docile still retain at least some of their capacity for aggressive behaviour and can be provoked to attack each other or bite their handlers in certain circumstances. The fact is that in a properly managed colony care is taken not to provoke such undesirable reactions. The remedy for savagery in a laboratory colony is not to use protective gloves or handling forceps, but to learn to handle the animals so as not to provoke defensive behaviour.

When arranging the conditions of confinement in the laboratory animal house it is, therefore, reasonable to consider the nature of the wild ancestor. Wild mice are photophobic and thermophilic, although from the point of view of temperature they are highly adaptable. They may therefore be expected to grow and breed better in warm dark boxes rather than in open wire cages, and this expectation is found in practice to be realized. Wild rats, though also largely nocturnal animals, are, however, more frequently around in the daytime and live a hardier life, and this is reflected in the ability of the laboratory rat to breed better than the mouse in open wire cages, and with less coddling; although the rat, being crepuscular in nature, still thrives better in a solid walled cage than in a wire one.

These considerations are more valid when the number of generations separating a laboratory colony from a wild ancestor is small. The point has been beautifully illustrated by McLaren and Michie (personal communication) in their attempts to breed in captivity a group of Gough Island mice (*Mus musculus*). They found that even in a large cage no breeding took place until, following the earlier experience of Professor L. C. Dunn in the United States with wild caught mice, they provided a large exercise drum, which the mice used with great enthusiasm. Breeding ensued. The wild mice roam freely

over the uninhabited Gough Island and it would seem that a great deal of exercise is a necessary prerequisite for successful breeding in this animal.

It should be stressed that optimum conditions in the laboratory animal house do not entail a slavish imitation of the supposed environment of the wild archetype. In any event, such a thing would seldom, if ever, be attainable and would certainly not be feasible. But it is suggested that, for successful laboratory breeding, rearing and management, consideration should always be given to the biological needs of the animal, and a clue to the detailed nature of these needs may often be found by reference to the archetypal environment. A detailed discussion of this thesis is contained in *Laboratory Animals Centre Collected Papers*, Volume 11 (1962) (especially the paper by Crowcroft), which reports the proceedings of a symposium on the environment of the laboratory animal.

The reader who wishes to examine further the consequences to the animal of a change from natural conditions to captivity should refer to Hediger (1950, 1955). Here, in a manner so far nowhere bettered, is an account of what in the author's view happens to the animal in these circumstances. Although Hediger is writing generally about wild animals kept in zoos, and especially about individual animals captured in the wild and subsequently confined in proximity to man, what he has to say is relevant to laboratory rats and mice that have been bred for many generations in laboratories. A reasonable first assumption is that the laboratory rat or mouse, being ultimately derived from wild ancestors, possesses ancestral characteristics; the onus of breaking down this assumption, piecemeal, rests on the laboratory worker, who must satisfy himself about the evidence for believing that what is undesirable in the wild archetype has been eliminated from the laboratory counterpart.

III. Requirements to be met in the Laboratory

If animals were chemical reagents, they could be kept in stoppered bottles on shelves and taken out for use as they were wanted. Since they are not, they have to be kept in cages that are compatible with life and health and comfort, but the cages can still be stacked on shelving in such a way that the regular needs of the animals—feeding, watering, cleaning, handling and the turnover of stock—can be conveniently met, and the animals are readily available for experimental use.

Animals are expensive, and their proper care entails a lot of labour. It is therefore only reasonable to design animal facilities so that, from the investigator's point of view, the nearest possible approach is made to the convenience of the chemical laboratory with its handy row of bottles, subject to the proviso that the biological needs of the animal are first assured.

Now, although laboratory animals cannot, like chemicals, be kept in stoppered bottles on the shelf, ready at all times for instant use, they are in

fact customarily kept on shelves, in cages, and it is these cages that constitute the *Lebensraum* of the animals they contain. Therefore, in seeking to provide the optimum conditions, it is the microenvironment within the cage that has to be considered. On the other hand, the room containing the cages provides the *Arbeitsraum* of those who have to do with the animals—call it, for contrast, the macroenvironment.

There is no reason to assume that what are acceptable working conditions for man will constitute the preferred environment for rats or mice. On the contrary, so far as anything definite is known about the preferred environment of these species, it seems that what suits them best would be unacceptable for man. At the same time, there is equally no justification for assuming that the microenvironment (within the cage) is identical with, or even closely similar to, the macroenvironment (in the rooms). There can be great differences between the two, depending on a number of factors.

A. FACTORS AFFECTING THE MICROENVIRONMENT

The barrier between the macroenvironment and the microenvironment (in the senses indicated) is the cage periphery. The more freely air and heat can permeate this barrier, the more nearly will the two environments approximate. Open wire mesh offers little or no hindrance to permeation, but even in this instance the animals within the cage will produce heat and material products of metabolism. These will escape into the room so long as there is a gradient from cage to room, which implies that there is a difference between the two environments.

Consider, however, a cage with solid sides and floor, of the conventional shoe-box variety. Such a cage interferes grossly with the free exchange of air, which can only occur through ventilation holes in the lid (or the sides, if they are perforated). Even the lid may constitute a further hindrance to free circulation, depending on whether it is of wire mesh, perforated sheet or, over part of its area, solid. Moreover, conduction of heat through the solid sides of such a cage will depend on the material of which the cage is constructed. Wooden cages provide the greatest insulation against heat, sheet metal the least, with glass and plastics somewhere in between.

Exchange of air, and with it the products of metabolism including heat, will also be facilitated by air movement within the room. Draughty rooms are as undesirable for the animals as they are uncomfortable for the human worker. Rooms with very little air change and a tendency towards stratification of air and the formation of stagnant air pockets provide poor cage ventilation and a stale atmosphere, which is equally undesirable for both animal and man. The ideal to be aimed at is a regular change of air in every part of the room, with a deliberately contrived mild air turbulence short of palpable draughts.

Finally, in considering the relation between *Lebensraum* and *Arbeitsraum*, there is the furnishing of the cage itself. Rats and mice are singularly adaptable

animals, which means that they possess efficient built-in mechanisms for regulating their microenvironment. The capacity of all homeothermic animals to maintain a constant *milieu intérieur* extends in most of them, and especially rats and mice, to an ability to regulate their microenvironment, given the means to do so. An animal placed in a bare cage, with no bedding or nesting material, is deprived of exercising this ability; the built-in mechanism is paralysed, so to speak, or, more accurately, the behaviour pattern expressing it cannot be released. It will still attempt to maintain its body temperature by conserving or creating heat (e.g., by piloerection or shivering) or losing it (e.g., by vasodilatation in the tail, a heat-regulating organ), but where the difference between preferred and ambient temperatures is great this may require a considerable effort on the part of the animal. Bearing in mind that in attempting to provide a uniform environment in the interest of uniformity of the animals it is a uniformly optimum environment that is needed, it is clearly undesirable to make demands on the heat-regulating mechanism that are both severe and prolonged. Better by far to provide bedding and nesting material that the animal can utilize to suit itself. In doing so, it will be able not only to arrange its microenvironment according to its needs, independently of considerable variation in the macroenvironment, but also to alter it according to the same needs. It will also have something to occupy its time, which may be important for animals whose vital needs are satisfied without any serious call on their own efforts. A spell in the extreme cosiness of the nest containing a new-born litter will, in these conditions, be relieved by a cooling off and an airing in the course of a visit to the food basket at the other end of the cage. As the litter grows, the nursery windows can be opened a little more. In this way, the animal, given the means, provides an optimum environment more nicely regulated than all but the most exacting mechanical contrivances in the animal room could ensure; better still, even, because it can vary it optimally.

Indeed, without exploiting the animal's own ability to regulate its microenvironment, it is hard to see how an optimum environment can be achieved. If the state of the room suits the animal, it will be unlikely to be acceptable to the human worker. Moreover, it cannot be assumed that an optimum environment is synonymous with an unchanging environment. It has already been suggested that animals may prefer to move from warm to cool at times. When the young begin to grow their fur and move about, periodic visits to the cooler end of the cage almost certainly assist good pelage, just as good feathering in young chicks is assisted by having a warm resting compartment and a less warm open run in the brooder.

B. THE MACROENVIRONMENT

Let it be assumed, then, that the preferred, and therefore probably the optimum, microenvironment for rats and mice is something markedly

different from that of the human worker. The latter also deserves consideration, and the provision of conditions of temperature and ventilation within the limits of the comfort zone will be appreciated. Is it possible to reconcile the optimum conditions of *Arbeitsraum* with those of *Lebensraum*? If the human conditions are acceptable outside the cage, can the *Lebensraum* within the cage be made acceptable to the animals?

1. Heating and ventilation

For most people an ambient temperature of 20-21°C (68-70°F), give or take a degree or two either way, is within the comfort zone, and this is easily attainable in a modern animal house without any very elaborate or expensive means of control. Although frequent changes of air are necessary in living rooms, spacious offices or workshops, the conditions in this context in an animal room resemble more closely a crowded office, or perhaps a cinema, and require from five to ten air changes every hour if staleness is to be avoided. Some authorities recommend even fifteen or twenty changes, but this would seem to be excessive in the absence of inconvenient overcrowding or indifferent sanitation.

This number of air changes is not, of course, governed by the need to replace oxygen and remove carbon dioxide. Even in a crowded animal room a fraction of one change an hour would suffice for this. The air needs to be changed to remove moisture and odours, and to dilute the bacterial and dust content of the air.

The means adopted to produce the required temperature and ventilation in an animal room will depend on the external climate. In temperate climates with no excessive variation between summer and winter, the provision of heat in winter, with little or no cooling in summer, may suffice. But in most parts of the world such simplicity will exact a penalty in temperature fluctuations outside the acceptable range, and even in the English climate there are sometimes spells of hot weather in the summer that will take their toll of comfort and efficiency in both man and animals.

The choice of methods of heating and ventilating is more the province of the engineer than of the biologist, who should be content to submit the following specifications to the engineer.

a. The normal temperature range desired, and the upper and lower permitted limits: taking into account the heat produced by animals and by those working in the animal rooms.

b. The number of air changes required—say six per hour. This must apply to every part of the room equally.

c. An ability to vary the temperature of each room, within the permitted limits, by individual room thermostats.

d. The differential air pressures in the rooms and in adjacent areas. For example, in breeding rooms, the pressure should be slightly higher than in the

corridors, so that leakage is always outwards, and there is less chance of airborne infection being sucked in. In experimentally infected animal rooms the reverse should be the case, in order to contain the infection.

e. What emergency precautions need to be taken, if any, in the event of a breakdown of the mechanical system.

The degree of sophistication of the heating and ventilating system will depend on what the biologist demands and how much he can pay for it. Full air conditioning is the best at which to aim, and for many purposes and in many climates nothing less will do. But, for reasons already explained, only rarely would the biologist be justified in demanding such a narrow constancy of temperature in all seasons, and such a high rate of air change, as would raise the cost of air conditioning unduly. Rats and mice are very adaptable, and the gentle exercise of this faculty from time to time is less likely to do harm than good. In general, overheating is more damaging than cooling.

2. Moisture

For rats and mice the relative humidity should not fall below about 50%. In the English climate this will seldom happen, except during spells of cold dry weather, which are usually of short duration only. In some countries, however, low humidity may be a serious problem and steps will have to be taken to raise it artificially.

A higher relative humidity, up to about 80%, is of small concern to rats and mice, but may make the macroenvironment unpleasant for human workers. Rats and mice accommodated at a reasonable density in an animal room will produce every 24 hours a volume of water vapour, from exhaled air and evaporation of urine, corresponding to a litre of water for every square metre of floor area. With several air changes every hour the removal of this water vapour presents no serious problem; but if it were to be recirculated, which is undesirable from other points of view, then it would have to be partially dehumidified, an expense that might well offset the economy of recirculation.

3. Odour

Most mammals and birds, including man, when confined produce characteristic odours, by exhalation and from the excrement they produce. Bodily exhalations may sometimes be aggressively noticeable: for example, the acetamide smell of male mice. The amount of such odour is of course directly proportional to the density of the animals in a given space, and since the density of laboratory animal colonies is normally likely to be high, exhalatory odour can often become a problem. There seems also to be an indirect relationship between the amount of odour produced and the state of health of the animals. Heavily parasitized dogs are notoriously more noisome than healthy ones, and the same is probably true of rats and mice.

It should, however, be remembered that these characteristic smells are of biological importance to the animals, in which the olfactory sense is well developed. With its aid they mark their territory—the cage—and their mutual recognition depends at least as much on the olfactory as on other senses. Bruce (1960) has recently shown that personal odours affect reproductive performance quite profoundly. If it were possible, therefore, to render an animal room completely odourless even to the animals, and maintain it so, the effect on the animals would be not unlike depriving them of sight; it would probably be more severe because the sense of smell is more important to them than sight. Nevertheless, even rats and mice can presumably have too much of a good thing, and those that look after them will certainly prefer that animal smells be kept within tolerable limits.

Here again the distinction has to be drawn between *Lebensraum* and *Arbeitsraum*. Transfer to a clean cage is a disturbing event in the life of a rat or a mouse. The animal's first task is to mark the clean cage with its own personal smell, which accounts for the busy manœuvres it immediately practises when it is so transferred. If by any chance the new cage or its contents, through inefficient hygiene, contains remnants of a strange smell left behind by a previous occupant, the manœuvres are all the busier, for the old smell must be masked if the new occupant is to settle down. This entails the increased production of odoriferous material, possibly from the preputial gland of the male, and from the urine of both sexes. It follows that inefficient cage cleaning will lead, from this cause in addition to others, to a smellier animal room, but it should not be overlooked that even the introduction of a water bottle from another cage (which should not take place in any event) may have a similar effect.

Some of the odour within the cage, which forms part of the preferred environment of the animal, will diffuse into the room, where its presence is not so welcome. Good ventilation will, however, deal with this quite adequately, provided the animals are not being compelled to overwork their odour-producing mechanism. Even the penetrating smell of acetemide in a mouse colony can easily be kept down to a level that is hardly noticeable, if both hygiene and general management are of a high order.

There is another source of animal room smell that contributes nothing to the well-being of the animals and is even more offensive to man. This source is the excreta of the animals, and the products of their decomposition. The chief offender here is ammonia, produced by the action of bacteria on urine, especially in the presence of an alkaline residue left in the cages after cleaning.

Such odours serve no useful purpose to animal or man, are avoidable and should be avoided. An ammoniacal smell in the animal room is a sign that the sanitation, or the system of cage hygiene, is at fault, and must be corrected. Cages which allow the accumulation of a wet mess of urine and faeces, with perhaps food, water and bedding materials thrown in for good measure, to

decompose with the copious production of ammonia and other offensive substances, are clearly of bad design and must be condemned. The use of bedding materials, such as sawdust, with poor powers of absorption, may also contribute to this undesirable state of affairs.

The difficulty may be avoided if the droppings from the animals can fall through the floor of the cage into a tray below, which is frequently changed; or on to a platform that is frequently cleaned down, or furnished with a generous sprinkling of highly absorbent material. Solid-floored cages should contain an absorbent material, preferably acid in reaction, that will dry up the excreta; for example, peat moss, paddy husk or certain agricultural wastes. In recent years some mineral substances have been developed that are highly absorbent and low in cost. They can be useful for the trays of wire-floored cages, but they may prove too absorbent, and so desiccating, if used in solid-floored cages, especially when in contact with newly born animals.

A balance has to be struck between the need to change cages and bedding materials frequently, in the interests of cage hygiene, and the undesirable disturbance to the animal that accompanies every change of cage. It is possible to maintain a mouse room entirely free of ammoniacal odour, and with a very low level of acetamide, and yet have a high density of shoe-box cages and changes no more than once in 2 or 3 weeks. On the other hand, it is equally possible to have a mouse room stinking of ammonia and acetamide even though cages are changed twice a week.

4. Dust

The movements of animals within the cage stir up clouds of dust particles and aerosols that form ideal vehicles for bacteria. Much of this particulate cloud will diffuse into the room and may pass from cage to cage, and it can carry infection with it.

The local air turbulence in and around the cage cannot be avoided, because it is a direct consequence of the animals' mobility. A high degree of turbulence within the room will facilitate the spread of dust and bacteria round it, but to counterbalance this a large number of air changes will dilute the cloud and thus mitigate its undesirable effects. On the other hand, too frequent air changes cannot be attained without palpable air movements even amounting to draughts, however carefully the ventilation ducts are sited and baffled. There has to be a compromise, and common experience seems to indicate that something of the order of six air changes an hour can achieve the best balance, provided that the air is introduced at a low level at one or several points and removed at high level, again at one or more points, at the diagonally opposite side of the room. This can produce a low degree of turbulence, enough to prevent stratification with its effect of uneven ventilation, but not enough to stir up clouds of dust.

A certain amount of dust dissemination is unavoidable whatever the design

of cages and the choice of bedding and nesting material. The latter, however, can influence the degree of dustiness to a great extent. Much more important is the proper choice of animal room routine. Tuffery (1958) has shown that the bacterial content of the air in the animal room, which is normally low, is multiplied by a large factor if cages are scraped or cleaned out within the room. He has also shown that the refilling of a water bottle that has been in use creates an aerosol, which may be bacilliferous. From this it follows that it is better to avoid cage cleaning or scraping out and the refilling of water bottles within the room. The proper procedure is to prepare clean cages, charged with fresh bedding and nesting material, and merely transfer the animals from used to clean: a fresh water bottle is also supplied at the same time. The used cage, with its content of soiled bedding, is then removed to the cage cleaning area elsewhere and dealt with. The old bottle is similarly treated.

5. Working conditions

Once the atmospheric requirements of the animal have been met, it remains to create a good human working environment. This offers few difficulties in regard to heating and ventilation because, in the light of the foregoing remarks, most types of cage and most methods of management achieving the best microenvironment for the animals are likely to create working conditions within the human comfort zone. Indeed, in these respects animal rooms should be very pleasant places to work in.

Good ventilation and heating, and more especially full air conditioning, are expensive. It is therefore necessary that the fullest use be made of the premises so equipped, by economical stacking of cages on shelves and the avoidance of wasted space. Given good atmospheric control, the limit of utilization of space is governed by accessibility of cages and the convenience of reaching them. Here opinions are so varied that no good purpose would be served by trying to lay down rules, and in any event the layout of animal rooms has been discussed and considered in numerous publications elsewhere (see page 20 for a selection of references on animal house design). But whatever way the racks of cages are arranged, there must be enough space for movement between them. A gangway of less than 1 m between banks of cages is impractical; 1·3-1·5 m is to be preferred. If the top shelf is more than about 1·6 m high, steps will be needed to attend to the cages on it, but for the sake of economy in space this may be considered a small penalty to pay for having a larger number of cages in a given room.

6. Light

The breeding performance of rats and mice is to a considerable extent dependent on the length of day; that is, the number of hours of illumination in the twenty-four. For this reason (as well as for others, such as the achieve-

ment of efficient temperature control) many rat and mouse breeding rooms are today built without windows, the illumination being provided artificially.

Very little is known about the exact amount of light and dark that produces the best breeding performance in rats and mice, but it is thought that 14 ± 2 h light and 10 ± 2 h dark is probably a satisfactory range. The preferred illumination may even be, not for a diurnal on-off rhythm, but for a permanent twilight. However, this is unacceptable for the human worker, who needs a good working light during the day and wants to save electricity at night. The quality of light does not appear to be important to the animals, whether daylight, tungsten filament or fluorescent lamp. Therefore, in the absence of any more precise knowledge of animal requirements, the duration of illumination of the animal rooms should be the same at all seasons of the year, controlled by a time switch, and it should be of a good working quality and intensity, governed by human considerations. Because rats and mice are somewhat light shy, however, too intense a general illumination should be avoided. Provision may be made for more local and intense illumination, for examining and sexing young animals, for example, by the installation of mobile or angle-arm lamps that can be brought into use when and where they are needed.

7. Noise

Rats and mice have an acute sense of hearing. In particular, some strains of both species are very audiosensitive, and may be provoked into convulsions by certain noises. The decibel level of noises generally, combined with their duration, is undoubtedly important, but so also is their quality. The persistent ringing of an electric bell will convulse mice of the DBA strain, and ram-jet engine noise of an intensity intolerable to man will convulse most rats within a minute or two. Yet even DBA mice can tolerate ordinary aircraft noise, whether from piston engines, propeller turbine or pure jet turbine, as experienced in flight. A recent trial arranged through the courtesy of the British Overseas Airways Corporation showed that such mice, as well as rats, guinea-pigs and rabbits, appeared unconcerned by the close proximity of a large jet plane revving up on the ground, which most human beings find hard to bear. They are equally unaffected by the noise in flight, from whatever type of engine.

It appears that explosive noises of high pitch are likely to be the most damaging. These include bell ringing, the hammering of metal on metal, and metal on stone or concrete. Such hammering can play havoc with rat and mouse breeding, and even a busy telephone bell in the animal house can be a serious disturbance to the animals. Hardly less desirable is the noise of metal equipment being banged about; metal cages on metal racks, or metal equipment generally being roughly handled. In view of this it is questionable whether metal cages have not serious disadvantages, at least in breeding

rooms, for it is almost impossible to avoid crashing them from time to time. Today, fortunately, cages made of plastic are coming more and more into use and for most purposes are likely to supersede metal ones, and among their many other advantages is the fact that they are quiet in use. Even the diehard advocates of wood are slowly being won over to them.

Some breeders quite firmly maintain that rats and mice breed better if they are provided with a programme of music, at least for several hours throughout the day. Their preference is said to be for the sort of humdrum unremarkable background music that is sometimes provided in factories and workshops for the relief of human tedium. Whatever rats and mice may think of it, music is sometimes liked by those doing routine work in the animal house, and there is no evidence that what is desired by them is in any way harmful to the animals.

C. HYGIENE

A full discussion of hygiene in the animal house is not strictly relevant to consideration of the physical environment, but it is nevertheless necessary to draw attention to certain points here.

Everything the animal comes in contact with—that is, all the materials of its physical environment—is a possible vector of infection. Whether an animal colony is conventional or so-called SPF (specific pathogen-free), it is equally important to ensure that the opportunities of pathogens getting in are reduced to a practical minimum.

Assuming that all modern animal houses are mechanically ventilated, if not fully air-conditioned, the least requirement is that the incoming air be filtered to remove dust and with it a large proportion of its bacterial load. Microfiltration to remove all micro-organisms, scrubbing with water, and sterilization by ultraviolet light or other means, should be considered, balancing the advantages of more thorough treatment against the increased costs of installation and upkeep.

Water, for drinking and for washing down and other purposes, may also require attention. Normally, mains water, rendered fit for human consumption by chlorination, is good enough for all purposes, but if there is any doubt about the reliability of mains water, then provision may have to be made for local sterilization. Filtration, chlorination, irradiation by various means, or deionization, are all methods to be considered, again with an eye on the increased cost and the advantages to be expected from the extra effort.

All equipment, of whatever kind, coming into the animal house is a source of danger and must be disinfected, both on initial introduction and on every subsequent occasion when it is brought in. This applies especially to articles that may have been exposed to contact with wild rats or mice outside.

An even greater source of introduced infection is the bedding and food. Both are particularly liable to micro-organismal contamination outside and

both come into intimate contact with the animals in the animal house and will be actually ingested by them. Sterilization of bedding and nesting materials should offer no great problem, for they can be autoclaved, or treated with fumigants such as ethylene oxide. (Allen *et al.*, 1962, have reported that wood shavings treated with 20% ethylene oxide at 60°C for 2 h under 20 lb pressure (1·3 atmospheres) can produce toxic glycols, although the experience of other laboratories, using 90% ethylene oxide at 21°C for longer periods, seems to indicate that this may be a safer procedure.)

Food presents a more difficult problem. Bruce (1953) showed that the diet she used would stand autoclaving, and this procedure has been shown to work elsewhere. Pasteurization, a less violent treatment, destroys vegetative pathogens but does not kill spores. However, for most purposes spore-forming organisms are of little or no importance in this connection. Irradiation is another method of sterilizing food, and when present technical difficulties have been overcome it might prove the method of choice.

Most of the raw ingredients of laboratory animal diets are almost certain at one period or another of their existence to risk being contaminated by vermin, and thus food is perhaps the greatest danger of all to the health of an animal colony. There is no reason why suppliers of foodstuffs should not offer laboratory animal diets, ready sterilized, in sealed containers, and this in fact is already being done in certain countries, notably the United States. Equally, sterilized bedding and nesting materials ought also to be available commercially.

Pests will infect any animal house to which they can gain access, and will carry in infection. All animal houses should be proof against them. Wild rats and mice and other macrovermin should be physically barred by suitable structural design. Arthropod pests can to a large extent be stopped physically at the peripheral barrier, but flying or crawling insects, if they do enter, can be dealt with by the judicious use of insecticides within. This may take the form of insecticidal lacquers on areas with which such pests are likely to come into contact, preferably on their way in rather than after they have gained entry; or on occasion by the use of insecticidal sprays or aerosols. Whatever method is used, it must be effective; the rule should be that any kind of pest is not to be tolerated within the animal house.

Lastly, all persons entering the animal house must be regarded as potential vectors of infection. No one who does not work there should be allowed into a breeding colony, which means that casual visiting is forbidden, even by members of the scientific staff who have no good reason for entering. The extent of the personal decontamination precautions must depend on local considerations. The least that can be demanded is a change of outer garment (for example, a white coat), stepping through a disinfectant foot tray and washing the hands. At the other end of the scale comes a complete change of all clothing and footwear, together with a shower on every occasion of

entering. (For germ-free work, which is not being considered in the present context, even more stringent precautions are necessary.) A new animal house being built today should take into account the need for the shower and change routine, and set aside a sufficient area for a decontamination suite, unless there are cogent reasons for being permanently satisfied with something less.

Short of erecting a perfect barrier against infection and thus creating germ-free conditions, the better the hygiene the better will be the result. Indeed, SPF conditions are essentially the practical limit of refinement of conventional hygiene, with the additional precaution of Caesarian derivation to break the chain of vertical infection. Consequently, every precautionary act, from the simplest to the most sophisticated, will contribute something to the health of the colony it concerns. Half measures are fully half as useful as the full treatment, and therefore well worth taking if nothing better can be arranged.

D. HANDLING AND GENTLING

So far, consideration has been given mainly to the more strictly physical factors of the laboratory animals' environment. There is, however, another aspect that is partly physical and partly psychological, and which has a profound effect on the health and welfare of the animals. This is the relationship that obtains between the animals and those who handle them. The latter may indeed be regarded as part of the physical environment, and a very important part. Anyone who has worked with colonies of laboratory animals will have observed that the behaviour of the animals and even their breeding performance can be considerably altered when a new animal technician takes over their care. If careful records are kept of such events as escaping, birth of litters or incidence of cannibalism, and correlated with changes of employment of staff, this impression will be objectively confirmed.

Generally speaking, the more rats and mice are handled, the tamer and quieter they become, and a quiet animal is likely to breed better and grow more quickly than a nervous one. Moreover, any colony needs to get accustomed to the individual technicians who care for it, and a change of personnel is always a more or less disturbing event. People vary in their ability to handle these species well, but, as in any other field, a latent aptitude can be developed by training, often to a high degree. There are right and wrong ways of handling rats and mice, and insistence on acquiring and practising the right techniques will not only lead to better objective results, but is also demanded for purely humanitarian reasons.

Rules are notoriously misleading in such a clinical field as animal care, but one rule that is worth stating is this. If a person is bitten by a rat or a mouse, it is his own fault; had he been more skilful, he would not have been bitten. If there are any exceptions to this rule, it should be a point of honour not to invoke them. A person who is bitten should rather examine himself and ask

what he did to provoke a normally docile animal into aggressive behaviour. From this it follows that the use of handling forceps or gloves for rats or mice can never be justified on the grounds of personal protection against the trauma of bites; on the contrary, their use should be condemned, because it will almost certainly result in a colony of savage animals.

Time and trouble spent in learning how to handle rats and mice, and in gentling them so that they show no disinclination to be picked up and examined, are certain to be repaid. This is true of all strains, even or more especially of those with a bad reputation for savagery. On the other hand, if for reasons of hygiene or safety (for example, the risk of contracting a dangerous infection from an infected animal) forceps or other handling devices have to be used, a penalty will have to be accepted of having less docile animals.

IV. The Animal House

A. THE BUILDING

The foregoing discussion has given some indication of the environmental requirements that have to be met in a modern animal house for rats and mice. It is necessary now to translate this into terms of bricks and mortar.

The emphasis all along, as throughout the whole of this book, has been on breeding, rather than on accommodation of animals under experiment, although much of what is needed for one is equally necessary for the other. Nevertheless, there is no such thing as the ideal animal house, because what is ideal for one purpose will not necessarily suit another. It is for this reason that it is not possible for an architect to be presented with the plans of an existing animal house that is reckoned to be functionally perfect and merely copy it on another site.

Much has been written elsewhere on the design and construction of animal houses. A cursory examination of existing plans will reveal a great variety of solutions to this problem, and no attempt will be made here to treat the problem of planning in detail. It is for this reason that the consideration of what is needed, both by the animals and by the scientist who uses them, has been given at some length. A good architect should be made aware of the sort of environment that he is required to create; how he achieves this is for him, with his own specialist knowledge, to work out in relation to the site, the size, the availability of materials and the limitations of his budget. It is, perhaps, interesting to add that an architect's preliminary plan will never be perfect. It may be taken as axiomatic that it will fall short on some essentials and be unnecessarily extravagant in other respects; and this implies no reflection on a conscientious architect. It is the scientist's job to brief him as accurately and completely as possible. If the scientist fails in this, he cannot blame the architect for building him a bad animal house.

Most of the literature in the English language on animal house design and

conditions up to 1959 has been indexed and abstracted in *Federation Proceedings* (1960, 1963). Lane-Petter has summarized much of the existing information in Chapter 8 of *Provision of Laboratory Animals for Research* (1961) and in the *UFAW Handbook on the Care and Management of Laboratory Animals* (1957). Much useful material has been published by the Institute of Laboratory Animal Resources, U.S.A.; the Animal Care Panel, U.S.A.; the Animal Welfare Institute, U.S.A.; and the Laboratory Animals Centre, U.K.; to all of whom may be addressed questions on specific design problems. Articles describing individual animal houses have also appeared in the *Journal of the Animal Technicians Association* (U.K.) and in *Laboratory Animals Centre Collected Papers*, U.K. (see especially vol. 2, vol. 7 and vol. 11). Reference may also be made to the *Proceedings of a Symposium on Research Animal Housing* (1963) published by the National Academy of Sciences—National Research Council. The Symposium was organized in November 1962 by the Institute of Laboratory Animal Resources.

Some useful comments on the design of SPF quarters for rats are to be found in Chapter 5. Much of what is today regarded as somewhat specialized, because of the exacting requirements of SPF colonies, will no doubt shortly be regarded as standard practice for most laboratory animal houses. This prediction should be taken into account in the planning of any new animal house facilities.

Despite the copiousness of the above sources, no author has yet succeeded in codifying all the available knowledge and presenting it for universal adoption. Probably the best way of building a new animal house is as follows.

1. Settle on the site, the function and the available budget. 2. Choose the architect and brief him accurately about detailed needs. 3. Ask the architect to visit at least three or four other animal houses, where he may see methods in operation, and learn some of the things to copy and to avoid. 4. Study preliminary sketch plans.

At this state it is of inestimable value to seek further criticism of the plans, for the original planners may have got so close to the problem that they cannot see it in the round. Therefore 5, show the plans to (*a*) the scientist who is going to have the main charge of the animals (unless he has already seen them, as he should have done); (*b*) the senior animal technician who will be working in the animal house (who should also have been already consulted); (*c*) an outside expert, who will probably want to study the plans on the site, and discuss them with all concerned. 6. Have the plans redrawn and worked up in detail.

If this procedure, or something like it, is adopted, and all those who will use or work in the new animal house are consulted and kept constantly informed about what is being done, it should be possible to draw up working plans and specifications for the contractor that will produce a successful result. It should also be possible to avoid substantial alterations in the course of

building, which are expensive, frustrating and lead to serious delays in completion.

A few final maxims may not be out of place.

Not more than about half the total floor area can be made available for accommodating animals. The rest will be taken up by corridors and service rooms.

There is never enough space for storage, especially of cages not in actual use.

Machinery for washing cages and bottles, and for certain other routine purposes, normally represents an economy in labour and eliminates some of the less pleasant tasks in the animal house.

The creation of aesthetically pleasing conditions will result in better morale, which encourages better discipline and improved efficiency, as well as attracting better technicians.

Finally, coming full circle, the animal house has to house animals. It will perform this function to better effect if their biological needs are met as completely as possible, consonant with scientific requirements.

B. CAGES

It has been pointed out that the cages constitute the *Lebensraum* of the animals. Current ideas on cage design for rats and mice are very fluid, but certain preferences are beginning to appear.

Both species breed and maintain themselves well in cages with solid sides, with the top, sides or floor perforated for ventilation. Shoe-box cages for either species have solid floors, and a perforated or wire mesh lid for ventilation. Such a lid may also serve the useful purpose of providing a means of exercise, for both rats and mice will spend a lot of their time climbing about underneath it. Alternatively, when suspended cages are preferred, to be housed in batteries on the chest of drawers principle, the underside of each shelf constitutes a solid lid, while the floor of the cage is of wire mesh, allowing urine and faeces—but not new-born young—to fall through on to a tray beneath. If such cages are used for breeding, a nesting box or platform will be necessary for pregnant and nursing does.

The size of cages for rats and mice is governed more by convention and practice than by any substantial scientific evidence. Most people would agree that 12·5 cm × 30 cm × 11 cm high are adequate dimensions for a cage to house a pair of mice and their litter up to weaning, and from two to three times this area, 15 cm high, for a pair of rats. Such cages could house three or four mice or rats respectively (see also Lane-Petter, 1957).

For caging rats and mice other than for breeding, that is, as stock animals, larger cages will usually be necessary. Here the density limits already indicated should not be exceeded, and the tendency to put too many animals in the same cage, however large, is to be avoided. For transporting or holding large numbers it may be convenient to have groups of fifty or a hundred, but if as many as this are caged together they will crowd into a compact heap, and

those underneath may be crushed or suffocated. Twenty-five mice or rats in a single cage should be regarded as a reasonable upper limit.

Young rats and mice—that is, up to about 8 weeks of age—will normally live together amicably in the same cage. But when they reach sexual maturity the males especially are liable to fight, and may have to be separated. It is sometimes possible to keep the males in small groups after sexual maturity, if the cage is large enough and the cohabitants have become accustomed to each other, but this almost certainly puts a strain on their interpersonal relationships. Moreover, a social grouping is likely to arise that demonstrates a hierarchy, exposing the individual animals to different influences, and this will have a more or less profound effect on the animals, depending on their rank in the hierarchy. Nevertheless, for economy in space and cages, groups of four males or females may be tried, and if they settle down amicably no great harm is likely to arise.

Similar variable influences may affect small groups less liable to outright fighting. Chance (1957) has shown that responses of rats to drugs and hormones may be related not only to the size and design of cages but even more to the number of animals in a cage. Once again the animals demonstrate that they are highly complex systems and not just test-tubes on legs.

The materials from which cages are made have already been mentioned. Wood is a good thermal insulator, is cheap, can be sterilized by all methods except autoclaving, is moderately durable, and quiet and light in use. Aluminium, or its alloys, like all metals, is a poor insulator and noisy in use, is rather expensive, can be sterilized by any means, is normally durable but may be subject to corrosion. Galvanized steel is less expensive but heavier than aluminium, and shares most of the other qualities of aluminium. Stainless steel is expensive, very durable, but otherwise resembles the other metals. Glass is heavy, fragile, but easy to clean and sterilize; it is also rather noisy, and not always cheap. Plastics are variable in quality. Most in use today are thermostable and will withstand autoclaving. They are light, durable (except for some types of fibreglass resin), and usually cheap (except for polycarbonate, which is rather expensive), have moderate thermal insulation and are quiet in use. They have, however, to be moulded, and this limits the choice of shape, size and pattern unless they are ordered in quantities of several thousands or a standard design is accepted.

On balance, nearly all the advantages seem to be with cages made from thermostable plastics, such as polycarbonate or polypropylene. As they become more popular, the choice and variety of designs will widen and the prices will come down ever lower.

V. Conclusion

The plan of this chapter on the physical environment of rats and mice has been to consider what the animals, as biological systems, need for optimum

health and well-being, and to build around them suitable accommodation that is also compatible with the needs of the scientist. Although these species, above all others, are highly adaptable, the extreme exploitation of this characteristic can impair their efficiency as laboratory animals, and probably detracts from their comfort.

An animal house built to provide the optimum environment will inevitably cost more than a cowshed; indeed, area for area its cost will be about the same as that of a laboratory.

In modern research institutions that for reasons of economy have had to forgo air conditioning throughout, it is interesting to note that the animal house has often been the one place where this service is regarded as indispensible, and where emergency heating and ventilating plant has been installed, if nowhere else. So far have ideas on this subject moved in recent years.

The aim in any animal house should be to provide conditions in which the animals are, so to speak, at peace with their environment; where their physical needs are adequately met, including their need for interest and activity. If this is done, then the animals will have the best chance of being normal animals and, if uniformity is desired and sought through genetic manipulation, the resultant phenotype will also show the maximum uniformity.

At the same time, the care of rats and mice of the high quality almost universally required today makes exacting demands on those who care for them. The animal technician is no longer a server of food and a scraper of cages, but a trained and skilled person. He will not be able to exercise his skill to full advantage unless the physical environment meets the biological requirements of his animals, nor will he be content to work in conditions that are not reasonably acceptable to him. Scientists are likely to get both the animals and the technicians they deserve. They will deserve the best if they provide the best *Lebensraum* and the best *Arbeitsraum*.

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FURTHER INFORMATION ON ANIMAL HOUSE DESIGN AVAILABLE FROM THE FOLLOWING SOURCES

Animal Care Panel, Inc., Box 1028, Joliet, Ill., U.S.A. (*Laboratory Animal Care.*)
Animal Technicians Association, c/o G. H. Findlay, 21 Glebe Road, Welwyn, Herts, England. (*Journal of the Animal Technicians Association*, issued quarterly.)
Animal Welfare Institute, 22 East 17th Street, New York, 3, N.Y., U.S.A.
Institute of Laboratory Animal Resources, 2101 Constitution Avenue, N.W., Washington 25, D.C., U.S.A. (*Information on Laboratory Animals for Research*, issued quarterly.)
Laboratory Animals Centre, M.R.C. Laboratories, Woodmansterne Road, Carshalton, Surrey. (*News Letter*, issued half yearly.)

Chapter 2

Feeding Rats and Mice

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I. INTRODUCTION

Feeds for laboratory animals are produced and distributed commercially, which is convenient but not without its pitfalls.

There is a great variety of compounded diets, all of which have been designed and tested in the laboratory. Unfortunately, many diets that have been proved satisfactory under laboratory tests have subsequently not stood up to large-scale manufacture and use. This is due to misinterpretation of formulae or to physical and chemical changes resulting from the various methods of compounding, storing and transporting.

This chapter deals with feeds, methods of feeding and the practical application of food in relation to good husbandry of laboratory rats and mice.

II. NUTRITIONAL REQUIREMENTS OF RATS AND MICE

A. GENERAL REQUIREMENTS

Nutritional requirements are determined and expressed by Abrams (1961) in the following terms: water, carbohydrates, ether-soluble extract, proteins, inorganic elements, vitamins and antibiotics. These can all be supplied from a large variety of natural foodstuffs, many of which have a limited geographical distribution. Each locality has its own peculiar variety of foods which can

be utilized in conjunction with other ingredients to produce a diet suitable for animals maintained under laboratory conditions.

Individual foodstuffs vary and may not contain all the necessary nutrients. While complicated formulae are neither necessary nor advantageous, a grouping of foodstuffs has to be seriously considered if the full nutritive requirements of the species are to be satisfied. Grouping of foodstuffs has the advantage of allowing for a variety of conditions, permitting a degree of elasticity, and does not lay undue emphasis on one particular ingredient that for various reasons may not be available.

Nutritive values of foodstuffs generally incorporated into some compound diets fed to rats and mice are given in Tables I-IV.

TABLE I

Cereals

	Composition (%) Chemical Analysis						Digestible Nutrients (%)					
											per 100 lb	
FEED	DRY MATTER	CRUDE PROTEIN	CARBOHYDRATES	OIL ETHER EXTRACT	CRUDE FIBRE	ASH	DIG. CRUDE PROTEIN	DIG. CARBOHYDRATES	DIG. OIL	DIG. FIBRE	STARCH EQUIV.	PROTEIN EQUIV.
Wheat	86·6	12·1	69·0	1·9	1·9	1·2	6·8	63·5	1·2	0·9	71·6	9·6
Barley	85·1	10·0	66·5	1·5	4·5	2·6	7·0	60·9	1·2	2·5	71·4	6·5
Oats	86·7	10·3	58·2	4·8	10·3	3·1	7·2	44·8	4·0	2·5	59·5	7·6
Maize	87·0	9·9	69·2	4·4	2·2	1·3	7·4	63·7	2·7	0·8	77·6	7·6
Millet	87·0	10·5	60·7	3·9	8·1	3·8	8·0	45·5	3·1	2·7	58·5	7·7
Rye	86·6	11·5	69·5	1·7	1·9	2·0	9·6	63·9	1·1	1·0	71·6	—
Rice (polished)	87·4	6·7	78·0	0·4	1·5	0·8	5·8	75·8	1·1	1·0	71·6	—
Dari	88·9	9·6	71·2	3·8	1·9	2·4	7·7	60·5	3·0	1·0	74·1	—

TABLE II

Oil Seeds

	Composition (%) Chemical Analysis						Digestible Nutrients (%)					
											per 100 lb	
FEED	DRY MATTER	CRUDE PROTEIN	CARBOHYDRATES	OIL ETHER EXTRACT	CRUDE FIBRE	ASH	DIG. CRUDE PROTEIN	DIG. CARBOHYDRATES	DIG. OIL	DIG. FIBRE	STARCH EQUIV.	PROTEIN EQUIV.
Cotton Seed, Egyptian	91·2	19·6	21·5	23·9	21·2	5·0	13·4	10·7	20·7	16·1	80·6	—
Cotton Seed, Bombay	91·5	17·9	29·0	19·4	20·0	4·3	12·3	15·0	16·8	15·2	74·3	—
Cotton Seed, Brazilian	90·6	21·1	25·0	23·2	17·0	4·3	14·4	12·5	20·1	12·9	83·7	—
Groundnut	94·0	26·8	17·5	44·9	2·6	2·2	24·1	14·7	40·3	0·2	131·4	23·1
Linseed	93·0	24·2	23·0	36·5	5·5	3·8	19·4	18·3	34·7	1·8	119·0	18·7
Rapeseed	93·0	19·7	18·1	45·1	5·9	4·2	15·9	8·4	43·0	1·4	130·8	14·9
Soyabean	90·0	33·2	30·5	17·5	4·1	4·7	29·5	20·8	15·8	1·7	78·9	27·8
Sunflower seed	93·0	14·3	14·6	32·5	28·2	3·4	12·9	10·4	30·9	9·5	96·5	12·0

TABLE III

Oil Cakes and Meals

FEED	Composition (%) Chemical Analysis: DRY MATTER	CRUDE PROTEIN	CARBOHYDRATES	OIL ETHER EXTRACT	CRUDE FIBRE	ASH	Digestible Nutrients (%): DIG. CRUDE PROTEIN	DIG. CARBOHYDRATES	DIG. OIL	DIG. FIBRE	per 100 lb: STARCH EQUIV.	PROTEIN EQUIV.
Cotton cake (decorticated)	90·2	41·2	26·5	8·0	7·8	6·7	35·4	17·7	7·5	2·2	97·0	68·4
Groundnut meal (undecorticated extracted)	92·4	31·8	29·1	1·9	25·3	4·3	28·2	20·0	1·5	2·9	84·0	44·4
Groundnut cake (decorticated)	89·7	46·8	23·2	7·5	6·4	5·8	40·6	19·7	6·8	0·5	98·0	73·0
Linseed meal (extracted)	88·2	35·7	33·9	3·1	9·0	6·5	30·0	27·2	2·8	4·5	96·0	63·7
Linseed cake (English)	88·0	29·5	35·5	9·5	9·1	5·2	23·9	28·5	8·7	4·5	97·0	74·0
Linseed cake (foreign)	98·0	32·3	32·2	9·9	8·7	5·9	26·3	25·8	9·1	4·3	97·0	74·5

TABLE IV

By-products

FEED	Composition (%) Chemical Analysis: DRY MATTER	CRUDE PROTEIN	CARBOHYDRATES	OIL ETHER EXTRACT	CRUDE FIBRE	ASH	Digestible Nutrients (%): DIG. CRUDE PROTEIN	DIG. CARBOHYDRATES	DIG. OIL	DIG. FIBRE	per 100 lb: STARCH EQUIV.	PROTEIN EQUIV.
Blood meal	86·0	81·0	1·5	0·8	—	2·7	72·7	—	0·8	—	100	62·9
Barley bran	91·8	5·9	51·8	1·3	26·4	6·4	—	—	—	—	—	—
Whitefish meal	87·0	61·0	1·5	3·5	—	21·0	550	1·2	3·3	—	58·9	—
Dried grass	90·0	20·3	41·9	5·8	14·0	8·0	16·7	36·0	3·6	11·3	94·0	65·7
Lucerne meal (flower bud)	91·0	22·3	36·4	2·9	18·0	11·4	15·9	28·4	1·3	9·5	91·0	50·1
Meat and bone meal	90·3	50·3	1·0	15·0	—	24·0	39·2	—	14·3	—	100·0	67·8
Dried whole milk	95·8	25·5	37·4	26·5	—	6·4	—	—	—	—	—	—
Dried separated milk	89·7	32·8	47·9	1·5	—	7·5	—	—	—	—	—	—
Oat bran	90·5	8·0	2·7	1·1	—	83·9	4·0	35·6	2·0	8·1	88·0	45·5
Wheat-germ meal	89·8	32·0	41·9	9·2	2·0	4·7	—	—	—	—	—	—
Broad bran	87·0	14·7	52·8	3·8	9·5	5·8	10·9	37·4	2·6	2·2	77·0	42·6
Dried yeast	93·7	41·5	41·4	1·0	0·2	9·6	35·6	34·1	0·4	—	68·3	—

These figures may be used as a guide to the nutritive value of certain compound feeds, providing one realizes that for any particular feed no single figure can be taken as the measure of its nutritive value.

Investigation into practical methods of providing rats and mice with their full nutritive requirements has been somewhat neglected. A close and

prolonged study of the metabolism of these animals has been undertaken, but only so far as synthetic or semi-synthetic diets or nutrients are concerned.

The information derived from the work of Orr *et al.* (1936) and the more recent work of Ranadive (1957) demonstrates that in 21 years of outstanding scientific discoveries no great advance has been made in the practical application of feeding rats and mice.

Figures and tables only assist in designing a diet which *should* provide the nutritive requirements of the species concerned; Ranadive showed that when faced with the inevitable, these could be applied successfully. She was forced by circumstances to adopt natural foodstuffs and mix them in the correct proportions to provide the nutritive requirements of an inbred colony of mice, and was prepared to apply experimental methods to sections of the colony, carrying out trials in order to find out which ingredients or substitutes were most beneficial. When one ingredient was in short supply she used many substitutes, some more profitably than others. The initiative displayed by Ranadive and the results she obtained are evidence of how to unite scientific knowledge with practical ability.

When deducing a biological relationship one should consider the relevant variables, namely the food, the animal, relationship and reaction between ingredients, the environment with its effect on the animal and on the food. When these matters have been studied in detail it will be realized that the present accepted uniform method of feeding rats and mice is inadequate and that concerted efforts will have to be made for the introduction of a practical satisfactory feeding regime using local commodities.

It may be presumed that the wild rodents find locally grown foodstuffs quite satisfactory, yet few scientists have studied the contents of the stomachs of such animals. Much is to be learned from the animal in its natural habitat, how it deals with seasonal variations, whether its feeding habits change with the seasons and the climate. It may be claimed that after generations of living under an artificial environment and being confined to a cage the laboratory rodent can no longer be compared with wild species. Even so, the wild species have survived and multiplied in alarming numbers under some of the most adverse conditions in all parts of the world. This would suggest that the rodent has utilized local-grown foodstuffs to the best advantage when allowed to practise its own selection of available supplies.

Rations designed for farm animals and the manner in which they are presented vary with the species, the age of the animal concerned, the type of husbandry and the location, yet laboratory animals have been condemned to a uniform type of feeding, irrespective of age, sex, condition or environment. This uniformity has been so universally accepted that it has been known for one country to import rat and mouse diet from another country, where the animals were maintained in an entirely different environment. The exporting

country rightfully claimed the diet concerned was a good one, but the claim could hardly be substantiated after the diet had been subjected to a period of storage, indifferent shipment and extremes of temperature, and finally offered to animals of a different strain being maintained in another environment.

This blind acceptance of standard formulae as the only method by which a diet can be designed to meet the full requirements of the species concerned is responsible for the unsatisfactory conditions existing today. The time is fast approaching when it will be necessary to state that a diet contains X, Y, Z nutrients and not A, B, C ingredients. This is now the practice with a number of commercially produced cattle and poultry feeds. The diet concerned may be known as X manufacturer's C meal, containing the given nutrients in the given percentages, no mention being made of ingredients.

B. SPECIES DIFFERENCES

A diet is designed for two specific reasons: to provide the species concerned with the necessary nutrients, and to utilize available local supplies. This may entail considerable analysis and eventually a few feeding trials because a certificate of analysis cannot guarantee that a food is non-toxic. Nevertheless, with a list of the nutrients essential for the species and a list of available ingredients with their chemical analysis it is a simple matter to design a diet.

The nutritional requirements of rats and mice are not vastly different. Cuthbertson (1957) found that little work had been done on the actual requirements of the mouse for mineral elements and amino acids. There is, however, overwhelming evidence to support the belief that rats and mice do reasonably well on the same diet. Bruce and Parkes (1949) studied the growth and reproductive performance of both species and recommended a complete cubed diet for rats and mice (now diet 41B). A diet suitable for more than one species is convenient for both the manufacturers and the laboratory, but is convenience good economics? It is certainly not always good animal husbandry. Lane-Petter (1960) discussing the cost of laboratory animals draws attention to the fact that while much has been published on the nutritional requirements of the common laboratory species, little account has been taken of the greatly increased needs during heavy breeding, lactation and rapid growth. With modern methods of intensive breeding borderline deficiencies may not become evident until the third or fourth generation. Such information can be obtained from well-compiled records, properly analysed and interpreted. Such records will reveal the adequacy of the diet, if comparisons are drawn between the productive performances of successive generations. They may also show that a diet adequate for one species is inadequate for another. We have found this in our own laboratory, where a diet quite suitable for a colony of inbred rats did not support rapid growth, production

and lactation in CBA inbred mice. In this instance production progressively declined with each successive generation. A feeding regime adequate for both rats and mice is at present generally accepted by the majority of laboratories. However, although experimental evidence has established strain differences in mice and rats, there is little evidence of a species difference. It is probably safe to say that until further evidence is available the mouse may be taken as the more sensitive indicator.

The requirements of the two species have been reviewed by many workers including Morris (1944), McCoy (1949), Brown and Sturtevant (1949), Worden (1957) and Cuthbertson (1957). All these workers found results similar to those shown in Tables V-VIII (after Cuthbertson).

TABLE V

Water-soluble Vitamins

Vitamin	Rat		Mouse	
	Quantity per kg food	Reference	Quantity per kg food	Reference
Thiamine	1·25 mg	Brown and Sturtevant (1949)	3 mg	Morris (1947)
Riboflavine	2·5 mg	Brown and Sturtevant (1949)	1·5 mg	Morris (1947)
	5·0 mg	El Sadr *et al.* (1940)	4·0-6·0 mg	Fenton and Cowgill (1947)
	2·5 mg	Coward (1952)	4·0 mg	Coward (1952)
Pantothenate	10 mg	Brown and Sturtevant (1949)	1·5 mg	Fenton *et al.* (1950)
	10 mg	Coward (1952)	3 mg as d- calcium pantothenate	Coward (1952)
Vitamin B_{12}	15-20 μg	Cuthbertson and Thornton (1952)	5 μg	Jaffé (1950)
	20-25 μg	Emerson (1949)	10-20 μg	Bosshart *et al.* (1950)
	Not required	Coward (1952)		
Nicotinic acid (amide)	None, unless diet deficient in tryptophan	Hundley (1947)	Probably not required, unless diet deficient in tryptophan	Cuthbertson (1957)
	10-100 mg	Cuthbertson (1957)	10 mg	
Pteroyl Glutamic acid	None, unless gut synthesis prevented	Asenjo (1948)	Essential	Nielson and Black (1944)
	0·6-2·0 mg	Darke and White (1950)	,,	Fenton and Cowgill (1947)
			1 mg	Cuthbertson (1957)
Biotin	None, unless gut synthesis prevented	Cuthbertson (1957) Nielson and Elvehjem (1941)	Essential for lactation 80 μg	Fenton and Cowgill (1947) Cuthbertson (1957)
	100-400 μg		Not determined	Coward (1952)
Pyridoxine	1-2 mg	McCoy (1949)	1 mg	Morris (1947)
	1·0 mg	Brown and Sturtevant (1949)	5 mg	Fenton and Cowgill (1947)
	1-5 mg	Sarma *et al.* (1946)		
	1·0 mg	Coward (1952)		
Inositol	Not required	Coward (1952)	10-100 mg	Wooley (1941) Spitzer and Phillips (1946)
	3 g	Cunha *et al.* (1943)		
	300 mg	Engel (1942)	Not determined	Coward (1952)
Choline	1-2 g	Glynn *et al.* (1948)	Essential	Mirone (1954)
	1·0 g	Conger and Elvehjem (1941)	1·0 g	Cuthbertson (1957)
		Engel (1942)	Not determined	Coward (1952)

TABLE VI

Fat-soluble Vitamins

Vitamin	Rat Quantity per kg food	Reference	Mouse Quantity per kg food	Reference
A	3000 I U	Rubin and Ritter (1954)	300 I U	McCarthy and Cerecedo (1953)
	400 I U	Coward (1952)		
D	300 I U unless Ca/P ratio < 1·0 or > 2·0 or P < 0·3%	McCoy (1949)	Not determined	Coward (1952)
	None when Ca : P = —	Coward (1952)	Not determined	Coward (1592)
E	30 mg	Rose and Gyorgy (1950)	20 mg	Cerecedo and Vinson (1944)
	30 mg	Coward (1952)	< 80 mg	Lee *et al.* (1953)
K	Not essential unless intestinal synthesis inhibited		Not normally essential	
	1 mg	Cuthbertson (1957)	1 mg	Cuthbertson (1957)
	Not required	Coward (1952)		
Essential fatty acids (as linolenic acid)	2-20 g	Deuel and Reiser (1955)	Essential	Decker, Fillerup and Mead (1950)
		Thomasson (1953)		
		Greenberg *et al.* (1950)	5-10 g	Cuthbertson (1957)
	10 g	Cuthbertson (1957)		

TABLE VII

Amino Acid Requirements of the Rat for Growth and N Balance

Amino acid	Reference	Requirement g per 100 g diet	Requirement per rat per day
L-Valine	Rose *et al.* (1949)	0·7	
L-Leucine	Rose *et al.* (1949)	0·8	
L-Isoleucine	Rose *et al.* (1949)	0·5	
	Womack *et al.* (1953)	–	11 mg
	Forbes *et al.* (1955)	0·4	
D- or L-Methionine	Rose *et al.* (1949)	0·6	
	Schweigert and Guthneck (1954)	0·22[1]	
	Womack *et al.* (1953)	–	14·6 mg
			3·2 mg[1]
L-Threonine	Rose *et al.* (1949)	0·5	
	Womack *et al.* (1953)	–	10 mg
D- or L-Phenylalanine	Rose *et al.* (1949)	0·9	
	Armstrong (1955)	1·2	
	Armstrong (1955)	0·6[2]	
L-Tryptophan	Rose *et al.* (1949)	0·2	
	Hundley (1947)	0·1[3]	
L-Lysine	Rose *et al.* (1949)	1·0	
L-Histidine	Rose *et al.* (1949)	0·4	
L-Arginine	Rose *et al.* (1949)	0·2	
Non-essential amino N	Rose *et al.* (1948)	To make 12·5% N × 6·25	

C. STRAIN DIFFERENCES

The feeding of rats and mice varies considerably from laboratory to laboratory and it has been found that a diet fed to one rat colony does not invariably suit another. This may be due to the fact that after many generations

in the environment of one laboratory a degree of inbreeding may have produced an animal differing considerably in nutritional requirements from another animal of the same original strain produced in a second laboratory in a different environment. These effects are even more evident in highly inbred mouse colonies. Certain strains will grow and reproduce satisfactorily on a given diet while other strains may grow satisfactorily but fail to reproduce and lactate, on the same diet. Blackmore and Williams (1956) found that

TABLE VIII

Trace Element and Mineral Requirements of the Rat

Element	Reference	Requirement
Ca	Hubbell *et al.* (1937)	4·5 g
	McCoy (1949)	5-6 g
		6·0 g
P	McCoy (1949)	3·5 g
	Hubbell *et al.* (1937)	3·5 g
		4·0 g
Ca: P ratio	McCoy (1949)	1-2·0
		1·5
K	Grijns (1938)	0·5 g
	Grunert *et al.* (1950)	1·8 g
		5 g
Na	Grunert *et al.* (1950)	0·5 g
		5 g
Cl	McCoy (1949)	500 mg
		3 g
Mg	Kunckel and Pearson (1948)	200 mg
		500 mg
Fe	Waddell *et al.* (1928)	10-50 mg
		50 mg
Cu	Hart *et al.* (1928)	5 mg
	Hundley (1950)	15-20 mg
		20 mg
Mn	Hill *et al.* (1950)	3-10 mg
		20 mg
Zn	Hubbell and Mendel (1927)	2 mg
	Todd *et al.* (1934)	1·6 mg
		4 mg
I	Remington (1932)	100-200 μg
		200 μg
Br	Essential for mouse: Huff *et al.* (1956)	50 μg
F	Doubtful requirement	100 μg
Mo	de Renzo (1953)	40 μg
		100 μg

mice fed on a particular cubed diet failed to rear their litters. The failure was greater in C3H mice than in three other strains fed on the same diet. We have observed that certain inbred strains cannot be maintained on the stock diet without supplements. This stock diet is adequate for a non-inbred strain, but quite inadequate for growth, reproduction and lactation in, for example, the CBA strain. It is evident, therefore, that there is not only a difference in the nutritional requirements of certain colonies of the two species, but also a

difference in certain requirements of various strains of the same species. Morris found that when he was establishing the vitamin requirements of the mouse, various strains responded in a similar way. We have found that after successive generations of intensive breeding on selected diets there is a strain difference in their general requirements.

D. REQUIREMENTS FOR MAINTENANCE, BREEDING AND LACTATION

Although the nature of a good breeding diet should be no different in its nutrient make-up from that of a well-balanced maintenance diet, it has been found by Goettsch (1948, 1949) that the maintenance requirement of a 300-g female rat is approximately half that necessary for growth and reproduction.

If the diet is an evenly balanced one, adequate for the growth of body tissue, it should be adequate for the additional stress of pregnancy and lactation, provided the female is given the opportunity of satisfying her appetite. During these periods the food consumption of the female will increase daily with the growth of the foetus and with milk production. The additional food consumption may not, however, provide the necessary nutrients, if the ration is on a bare maintenance plane.

Reproduction and lactation are two biological functions which demand from the feeding regime nutrients in excess of maintenance level for the female. These extra nutrients must, therefore, be supplied either by increasing the ration or by supplementation that, in itself, has little to recommend it. Supplements should only be used to provide the missing nutrients in an unbalanced diet, and be given in the correct percentages to form an evenly balanced diet capable of supporting pregnancy and lactation.

Lactation imposes a heavy demand on the female, even more so in modern animal husbandry, when the female is generally re-mated at the post-partum oestrus and very intensive breeding is practised.

It has been found that the size and the health of young rats and mice at birth are not adversely affected by a moderately low plane of nutrition. Balfour (1956) found, however, that young rats produced on a semi-synthetic diet containing 25% protein were 1 g heavier at birth than those produced on a diet which contained only 10% protein.

We have found that when the diet is a poor one the number of young born alive is considerably less than the number born alive on a good diet. This may be due either to a lowering of fertility or to an increase in the number of foetal resorptions. The mobility of the young is also affected and this inhibits the mothering instinct in the female, who is herself debilitated and therefore fails to clean and stimulate the young to suckle. The young are too weak to provide the necessary stimulus to induce lactation.

A low plane of nutrition has no adverse effect on the potential growth and development of the young, but it has on lactation following pregnancy. This does adversely affect the growth and development of the young until they

are old enough to eat solid food. A stunted animal rarely develops to its full potential and under such conditions pre-weaning losses are very heavy.

During our own investigations on the adequacy of compound diets, we have observed in the breeding records of mice on suboptimal diets that the first litters are produced and reared satisfactorily, and that successive litters fall into one of the following categories.

1. Number of prenatal days for the second litters are considerably increased (although post-partum mating is allowed) and may range from 35 to 42 days; the oestrous cycles are normal but the female fails to conceive.

2. Litters are produced normally but the second litters are seldom reared; this also applies to the third and fourth litters.

Another evidence of a suboptimal breeding diet is the shortening of the economic breeding life of the female. These observations are only possible in a fully recorded colony, which may not be practical in large breeding units, but such units should maintain a small fully recorded primary colony from which their breeding stock is drawn. The records should be analysed to provide information on the adequacy of the breeding diet and the fecundity of the stock.

The economics of an animal division are often based on the cost of foodstuffs, which is quite a fallacy when one considers the small amount of food eaten by rats and mice. The true economics of an animal division, and a reliable indication of an adequate breeding diet, is the number of viable young produced by a given number of animals in a given area over a stated time.

III. Foundation of Feeds

A. Discussion of Ingredients

A feed should contain ingredients which will provide the necessary nutrients in the right proportions.

When estimating nutritive values of diets one must bear in mind whether the essential nutrients are given as percentages of the complete diet, or whether they are given as the absolute amount required per animal per day. The former normally applies to the proteins, the carbohydrates and the fats, but the latter generally to vitamins and minerals. This may lead to complications, for when the essential nutrient is given in anything other than percentage of the complete diet, the inevitable question arises, do the given figures apply irrespective of the condition of the animal or the amount of food it consumes? Cuthbertson (1957) deals with nutrients in a more practical manner by either giving the percentage or the amount per kilogram of diet.

By using analytical tables, much can be done to design diets and to adapt compositions to meet exigencies. Chemical analyses do have limitations but they also have their uses, as shown by Ranadive. Worden (1957) wrote: "The limitations of so-called proximate chemical analysis in defining the nutritional

value of a diet are, of course, apparent to all who are concerned with nutrition."

In the compound feedstuffs slight variations in composition are frequently unavoidable in the light of the supply position, and alternative formulae are usually to be had if one or more of the raw materials is not available.

The formulae of stock diets are numerous and varied; few have been designed to utilize ingredients peculiar to local conditions and even fewer have incorporated a wide variety of foodstuffs. The evidence of an overabundance of one particular ingredient in many British diets may be due to the limited variety available. It should, however, be borne in mind that a large variety of ingredients is preferable, thus reducing the effects of variability of any one of them. Plouvier (1957) draws attention to the undesirability of using a large percentage of one ingredient, where for example X, a characteristic of a cereal, has escaped analysis. The variant X will be reflected in the finished product, but the variation will be considerably reduced or compensated for if the finished product contains low percentages of several cereals, rather than a high percentage of one.

Thomson (1936), a pioneer in compound diets for laboratory animals, included twelve ingredients in a rat diet which required supplementing with fresh whole milk and green food. Dean *et al.* (1961) drew comparisons between five commercial feeds in mouse production. They obtained the best results from a diet containing fourteen ingredients, plus numerous supplements. The Rockland rat and mouse diet contains twenty-four ingredients, while Bruce and Parkes (1949) included six ingredients in their complete diet for rats and mice. The problem is whether to choose a diet containing a number of ingredients such as that recommended by Rockland or to choose the more simple formula of Bruce and Parkes.

Abrams says that unless time and materials are to be wasted in pointless calculations, a ration needs to be produced (*a*) for animals of a given type, (*b*) for set circumstances and (*c*) from specific foods. The problems vary from one species to another but the methods of calculation on the whole do not. He does, however, criticize the blunderbuss mineral supplements more beloved of salesmen than of nutritionists. The construction of diets must, therefore, meet the quantitative and the qualitative nutritional requirements of the animal. Nutritionists may say this cannot be done on a purely arithmetical basis, but it will be found that a composition made up from arithmetical and analytical tables is the normal procedure in the manufacture of cattle and poultry foods.

B. SPECIFICATION OF DIET IN TERMS OF NUTRITIONAL VALUE

Diets with a given theoretical composition could be produced if laboratories were to give the manufacturers more detailed instructions on the minimum nutritive standard of the diet concerned and also a list of desirable

ingredients with the permitted substitutes. Manufacturers should also produce on request a chemical analysis of each batch of manufactured diet, giving each a code number with the date of manufacture.

Thus any laboratory or group of laboratories in any one local area could standardize a diet by cross reference with each other and in consultation with the manufacturer. These references would not be concerned with analytical tables but with information on the behaviour of the colonies during the period when batch *x*, manufactured on a given date by manufacturer *y*, was being fed.

Plouvier, discussing the standardization of diets, found that it was impracticable to base the definition of a standard diet upon an analytical table and to adjust the formula according to these analyses, and that the definition of a standard diet cannot be expressed in the language of a chemist.

Standard lists of ingredients, classed as the essentials of a diet, are most misleading. Seldom do two samples of the same ingredient contain equal nutritive values, which vary to extremes and are controlled by environment during growth, harvesting, storing and processing. When computing a diet it is difficult to ignore the lists of feeding-stuffs that are known to constitute a good diet, but such feeding-stuffs should only be used when they have been checked to ensure that the nutritive value is equivalent to the given calculation. To work out a diet on a scientific basis for rats and mice, first deduce from the tables the total requirements of the animal concerned. Next, compute a suitable diet from the available foodstuffs, of which a working knowledge is essential. The final test must always be feeding trials within the colonies.

A universal standard diet cannot be envisaged, but a diet containing the standard nutritive requirements for any given species is possible through a closer co-operation between laboratory and manufacturer.

C. PALATABILITY

There are many ways of trying to determine whether or not rats and mice will, if given a free choice, show preference for one type of food over another. The reaction of rats to different foods will depend, very largely, on the treatment that the animals were given before being allowed free choice. This applies particularly to synthetic diets, plus or minus certain nutrients.

Little has been written about the palatability of practical feeds. We have observed, however, that certain cubes presumed to be identical with other cubes are not so eagerly consumed. Also that some cubes are more readily broken up, wasted and foraged through than others. This could be connected with either the texture of the cube or the fineness to which the various ingredients are ground. Palatability is not a fixed characteristic, especially in rodents who will eat almost anything when they are hungry. The modern methods of feeding rats and mice with a supply of somewhat uninteresting cubes which they have to manipulate through a series of bars to satisfy their appetite

could scarcely be used as a criterion of palatability. A sudden loss of appetite may not be connected with palatability but could be evidence of a deficiency of a nutrient which had been destroyed during preparation either chemically or through faulty storage.

Experimental studies have given little indication of a relationship between nutritive value and palatability; nevertheless the animal-nutrient relationship may be influenced by palatability.

D. METHODS OF PRESENTING FOOD

The methods of feeding rats and mice have undergone a considerable change since Thomson (1936) introduced a stock rat diet in cube form. He never claimed that it was a complete diet. Cube feeding was not, however, generally accepted in Britain until Bruce and Parkes (1949) introduced diet 41, now modified and known as diet 41B (Bruce and Parkes, 1956). These cubes have many advantages over wet sticky mashes and other concoctions which are still used in many animal houses, not because there is a sound scientific reason for using them but rather because they have become traditional.

Lane-Petter (1957a) discusses the disadvantages of cube feeding, drawing attention to the generally held impression that named diets are, in fact, standardized, and points out that it is wrong to assume that the composition of a compound diet is equal to the sum of the composition of the ingredients. The variation between these two quantities is dependent upon the country of origin of the ingredient, time of harvesting the crop, conditions under which it has been stored, method used for compounding, storage of the compounded diet, and chemical action which might ensue between the various ingredients at certain temperatures.

Rodents, being nocturnal animals, eat very little during the day time. Mashes therefore, even with daily feeding, are never fresh when eaten, especially at week-ends and during the holiday periods when the animals are fed early in the day. The washing and sterilizing of feed pots, the daily removal of stale food, the frequent cleaning of cages, are all time-consuming, but unnecessary when the diet is fed in cube form. When fed in specially designed containers, cubed diets reduce waste to a minimum and eliminate the time and labour expended on the preparation of other forms of feed that must be supplied fresh daily.

Parkes (1946) recommends the following type of rat and mouse cake container: "Cube containers for mice should have vertical bars placed $\frac{5}{16}$ in apart and for rats vertical bars placed $\frac{5}{16}$ in apart secured by tie wires 1 in apart."

There are several other types of container, but in general the Parkes principle applies. The bars must be placed in such a manner as to allow the animal access to feed at will, yet prevent it from pulling the cubes through

the bars of the container into the cage. The design and positioning of the containers are of some importance. They must be placed in a position which will allow the smallest and weakest animal to reach the food, yet be absolutely clear of the floor of the cage. Long deep containers have a decided disadvantage; both rats and mice crawl up the bars urinating on the cubes as they do so, thus making them unpalatable.

Ventilation within the cage and the design of the container must also be considered. We have found that containers placed inside certain mouse batteries have a marked disadvantage, because the cubes become mouldy very quickly due to the high humidity within the cage and poor ventilation round the containers.

Fabrication of the cube is also important. It has been our experience that a soft fragile cube entails considerable waste, but we also found that the young animals grow better on such cubes because the parents pull particles of the cubes through the bars of the container and drop the particles on the floor, thus providing the young with a liberal supply of food during the week or so before weaning. A very hard cube prevents this and reduces waste to a minimum, but young and weak animals have difficulty in manipulating sufficient food through the bars to support their normal growth and development.

Fabrication of the cube, the methods of compounding, packing and storing still require further investigation; such investigations are now being undertaken at the Laboratory Animals Centre.

It should not be assumed, however, that cubed feeding is the only accepted method. Good animals are being produced at the Sprague-Dawley Farms, Wisconsin, where the feed is freshly ground and mixed on the premises. An automatic device provides the animals with a constant supply of fresh meal. Fresh drinking water is also provided through an automatic system. Automation has not adversely affected the colony; the animals are prolific, grow fast, are reasonably healthy and exceptionally docile.

The majority of commercial breeders in Britain still feed their mice a high percentage of local grown cereals. Several laboratories in Czechoslovakia feed their rats and mice a diet which is baked in the form of a cake. Sabourdy (1961) feeds rats and mice on a baked biscuit. One British laboratory producing the largest rats in the country has a feeding regime whereby a menu, containing numerous natural foodstuffs, is given in rotation throughout the week. This may not be ideal but the quality of animal produced suggests that it has much to recommend it.

E. METHODS OF PRESENTING WATER

Lane-Petter (1957b) in the UFAW Handbook deals extensively with watering equipment for all species of laboratory animals. He states that some rodents exist on a very small water intake and that where the diet is moist, as

with mash, or contains green food or roots, the water requirements of the animal may be satisfied without the need of extra water. However, unless it is certain this is the case and there are no special reasons for not giving water, it should not be withheld.

The drinking habits of the two species are somewhat different; mice can drink from an open pot without excessively fouling it, but rats scoop up the water in their front paws and foul it almost immediately.

In conventional colonies the risk of contamination and cross-infection is always present. Open drinking pots are, therefore, not recommended and fountains, such as are used for watering poultry, are also unsatisfactory for similar reasons. Inverted bulbs or bottles fitted with a spout or canula are in general use and they are still one of the most practical methods of supplying water to rats and mice. There are several types of bulbs and bottles, the choice of which will be governed by the design of the cage, the species of animal and the number of animals housed in one cage. Water consumption will be approximately 1 ml of water per 1 g of dry food consumed. Lactating females drink considerably more. Allowances must also be made for young animals, who start drinking before they consume appreciable quantities of dry food. Water containers should be large enough to hold several days' supply and, though it is not generally accepted, they need not be filled daily and should never be topped up. A fresh sterilized bottle should always replace an empty one.

Filling devices for water bottles have much to recommend them, especially in large colonies of rabbits and guinea-pigs. We have found that it is more practical and certainly more hygienic to have each animal room equipped with a duplicate set of water bottles. Each water bottle holds several days' supply and no topping up is permitted. Arrangements are made to remove all the bottles on certain days of the week and to replace them by clean sterilized bottles filled with fresh water.

Tuffery (1959) in an investigation concerning mice experimentally infected with *Salmonella typhimurium* stated that there is a real risk of water being a vehicle of cross-infection, and he found that it was as much a problem of animal house management as of bacteriology. He draws attention to the importance of the correct method of filling the water bottles in order to reduce the risk of cross-infection. Bottles, drinking tubes and other fitments should be sterilized, either chemically or by autoclaving. During refilling the mouth of the bottle should not come in contact with the water tap nor be touched by hand, and when testing the bottle to ensure it is functioning, the finger tip should not be applied to the end of the drinking tube.

Automatic watering devices

In large colonies and under certain circumstances, automatic watering devices have much to recommend them. There are several kinds of automatic drinkers, but all have certain features in common. The cages and equipment

must be specially designed, particularly cages with solid floors. The system may be connected either to overhead water storage tanks or to the mains water supply, but a constant head of water must be maintained. A fluctuating pressure may cause stoppages, leaks or flooding, and excessive pressure frightens the animals and prevents them from drinking.

Ash (1960) describes the mechanics of one system, where the water is supplied from a vertical series of reservoirs filled from the mains and providing an equal and constant head of water. The animals drink from small brass drinking founts which have been placed in the framework of the batteries of cages and designed so as not to interfere with the opening of the cages. He draws attention to an interaction between the metals used in their construction and the water, whereby a white deposit collects on the founts and prevents them from operating. The mechanics of all automatic systems present certain difficulties, particularly the cleaning and flushing of the system and the unscrewing and sterilizing of the founts when the occupants of the cage are being changed.

Free access to drinking water must be provided for all animals at all times, but how it is provided will be governed by local conditions.

IV. Preparation and Storage of Food

A. KEEPING QUALITY OF FEEDS

In order to preserve the nutritive value of ingredients and compounded diets, food preparation and storage rooms should be designed to meet the needs of each laboratory. They should never be a section of the general stores. These rooms should be cool, dry, well ventilated, vermin proof and protected from sunlight. They must be designed to permit complete sealing for fumigation as and when necessary. All doors and windows should be made fly-proof and in every room aerosol generators installed. A high standard of hygiene should be maintained inside and outside the rooms.

For ingredients normally stored in sacks, the rooms must be fitted with racks designed to hold the sacks in a manner allowing free circulation of air. In several establishments small bins are favoured. They are filled at the time of diet delivery, clearly marked and stacked. Food is delivered to the animal rooms in these bins which are cleaned, sterilized and returned to the food store when empty. Large bins are not recommended for food storage, irrespective of how they are constructed, because they are normally fixtures and therefore cannot be properly emptied, cleaned and sterilized, and because there is a tendency for them to be continually topped up with fresh supplies. In the most favourable storage environment the keeping quality will depend on the ingredients themselves. Compound diets should never be stored for more than short periods because of the real danger of deterioration of essential nutrients.

B. HANDLING OF FEEDS

The nutritive value of feeds, as defined by proportions of ingredients in a given formula, is normally adhered to by the producers. Both raw ingredients and the compounded diets may, however, be seriously affected by mishandling on the farm, in the course of manufacture, or during delivery. Since mishandling causes deterioration before delivery to the laboratory, it may be necessary to analyse chemically each batch of food after delivery.

1. On the farm

Cereals are usually damaged by lack of protection from the weather, delays in transport and bad storage, all of which will adversely affect the nutritive value of the feed. This should not, however, affect the finished product, because the manufacturer will have made chemical analyses on the cereals delivered and therefore will know their nutritive value and should correct for deficiencies.

2. *During manufacture*

Feeds with an unusually high moisture content must be stored in properly constructed lofts. They should be turned in the prescribed manner at regular intervals, otherwise moulds develop and grains begin to sprout. Storing in overheated mills before grinding and compounding is also a possible cause of deterioration.

The correct weighing, mixing and handling of the ingredients which goes into the compound diet is of the utmost importance and must be carried out by a competent person who will ensure that it will provide an evenly mixed diet containing the nutrients as dictated by the formula. After compounding, the cubes are very hot; they must, therefore, be allowed to cool before being packed. Failure to do this will result in deterioration.

3. Wrapping used by manufacturers

Hessian sacks are in common use but have the disadvantage of being very porous, thus the contents may become contaminated, for example, by placing filled sacks on wet, dirty floors or trucks. The contents can also be soiled by cats, dogs, wild rodents or other vermin. Sacks have the advantage of allowing air to circulate within them, thus reducing the moisture content to a minimum and greatly increasing the keeping properties. Waterproof paper bags consisting of several layers of paper provide a good means of protection against contamination and also against deterioration from the effects of external damp. When sewn up, these sacks are sealed and condensation of water may therefore occur inside them if the cubes have not been properly cooled and dried before packing. If such condensation does occur, the cubes quickly become mouldy.

4. En route

Manufacturers and shipping agents must ensure that diets *en route* are properly handled and adequately protected from the elements and from vermin. During transit sacks of diet must not be stored in heated rooms or holds, and they must be kept off dirty and damp floors and decks. Further, the cubed diet is somewhat fragile, therefore sacks of diet should not be handled in a manner which will reduce the diet to powder.

5. On receipt at the laboratory

Careful and intelligent handling is necessary on delivery. For those diets delivered in paper sacks, the removal of one wrapping or layer before transfer to the food store improves general hygiene. Other routine checks made, after clearly marking the delivery date on all sacks, should note the condition of the diet and remove a sample for analysis.

C. STORAGE PROBLEMS

It is generally appreciated that laboratory animal feeds may be contaminated with the excreta of cats, dogs, birds, wild rodents and other vermin. Such contamination may have occurred before the raw ingredients have been harvested or possibly whilst in store or during transport. It is possible, however, for foodstuffs to be contaminated before, during and after processing.

While every effort is made to ensure that foodstuffs are not contaminated, it must be recognized that contamination by chemicals, micro-organisms, parasites and insects is an ever-present hazard which must be effectively dealt with if the health of the colony is not to suffer. Spiegel (1961) carried out bacteriological tests on samples of feed taken from each batch as and when delivered. These tests may not be absolute proof of the absence of pathogens; nevertheless, such tests are of the utmost value and should be carried out whenever possible.

1. *Chemicals, toxic substances and moulds*

Bloom (1956) draws attention to the benefits of antibiotics in animal nutrition, especially in the feeding of pigs and poultry. Scientists have been most emphatic in demanding that antibiotics should not be used in laboratory feeds. Nevertheless, manufacturers use the same machinery and equipment for making pig and poultry feeds as for making laboratory animal feeds, so that the risk of contaminating a batch of laboratory animal feed with an antibiotic which had been added to the pig and poultry feed is very grave indeed. Mixing machines, conveyor shutes and cubing machinery cannot be guaranteed free from an antibiotic even after the most careful cleaning.

Several workers have reported reproductive failures due to laboratory animal feeds becoming contaminated with diethylstilboestrol. Hadlow *et al.*

(1955) reported serious reproductive disturbances including continued oestrus and scrotal hernia. Wright and Seibold (1958) reported irreversible sterility in a guinea-pig colony for similar reasons. Paterson and Brimblecombe (1959) reported hexoestrol contamination of mouse and rat cubes which caused a large number of abortions in their mouse colonies and also induced persistent oestrus in young stock. The contaminant was accidentally added to the laboratory animal feed at the rate of 5 g/ton. It is obvious from these reports that laboratory animal feeds should not be prepared in establishments where such contaminants are being used.

Groundnut meal, although not generally used in feeding rats and mice, has presented problems with other species of laboratory animals. Allcroft and Carnaghan (1961) drew attention to the toxic properties of certain groundnut meals, while Paterson *et al.* (1962) found that groundnut toxicity was the cause of exudative hepatitis (oedema disease) in guinea-pigs. These authors state that toxic meals have been used for compounding diets in this country for more than a decade.

Aspergillus, a genus of ascomycetous fungi, includes several of the common moulds, some of which are pathogenic. The species most likely to be found on laboratory animal feeds of corn and grains are *A. flavus* and *A. niger*. The latter mould is said to cause ill-health in animals which consume grain infected with it. *A. flavus* produces a very potent toxin.

2. Pests and vermin

Food kept in store for any length of time may become infested with mites, moths or beetles, and food stores may be invaded by flies, cockroaches, wild rodents or cats and dogs.

a. Mites. Tyroglyphus farinae is perhaps the most common pest of stored food. Large numbers of mites very quickly appear, even when the conditions of storage are good. The early sign of infestation is a greyish dust lying about in corners, crevices or ledges that are difficult to clean regularly. Feeds infested with this mite develop an unpleasant musty smell and quickly become unpalatable. *Tryoglyphus castellanii* frequently infests foodstuffs with a high fat and a high protein content. This pest has been associated with dermatitis on the hands of persons handling heavily infested foodstuffs which have been allowed to get damp or have been stored in a damp atmosphere. *Goitieria fusea* is a small brown mite often found in large numbers on top of flour and other cereal products. It is often introduced by sweepings from floor cracks.

b. Moths. Moths as such do little damage, but the larvae do much. The most common is *Ephestia kühniell* (mill moth) whose caterpillars feed on flour and other cereals until they pupate. Evidence of infestation is apparant from dead moths, empty pupae and masses of food webbed together.

c. Beetles. Beetles do not normally infest compound foods, but whole

grains are frequently attacked by *Calandra granaria* (grain weevil). Infested grain very quickly becomes hot, musty and unpalatable.

d. Flies. Flies do little or no direct damage to laboratory animal feeds. They must, however, be regarded as a source of infection, especially where they can travel between infected and non-infected areas and have access to open feeding pots and hoppers. The common house fly, *Musca domestica*, and the lesser house fly, *Fannia canicularia*, are the species most likely to be found in and around animal rooms, although the carcase flies, *Calliphora*, will frequent rooms where raw meat is being prepared or fed to animals.

e. Cockroaches. One cockroach seen should be regarded as evidence of an infestation. Every animal house provides favourable breeding conditions for the cockroach; nevertheless their presence should not be tolerated. They contaminate foodstuffs by their faecal matter, travelling considerable distances from one part of an animal house to another and carrying infection as they pass through infected areas to food stores.

f. Wild Rats and Mice. Foodstuffs are not only devoured by wild rats but are also contaminated by their faecal matter. In Chapter 3 of this book Tuffery deals with infections transmitted through foodstuffs. As already stated, contamination may occur while the foodstuffs are still in the field, the store, the mill, or during transport, over none of which the laboratory has much control.

g. Cats and Dogs. Contamination caused by these animals is, as in the case of rats and mice, mostly due to faecal matter. Tuffery also deals with infections passed on to rats and mice through food being contaminated by cats and dogs.

D. STERILIZATION OF FOOD

Provided that a high standard of hygiene is maintained in the food store and that these rooms are effectively sealed against the entrance of pests, the necessity to sterilize may be restricted to the food ingredients. Sterilization should be of such a nature that it will destroy bacteria, fungi, insects and parasites. It should be emphasized that in feeding germ-free and pathogen-free animals the food must be sterile. The process must, however, not be such that uncontrolled vitamin losses occur, or that the nutritive value of any other foodstuff is seriously damaged.

1. Pasteurization

The method has not been used to any great extent with laboratory animal feeds. Davis (1956) found that exposure to a temperature of 60°C for a few minutes killed all stages of common grain pests. The diet should be subjected to a temperature of 70°C for at least 1 h, and the recommended temperature must completely penetrate the mass of diet being treated. This would, undoubtedly, have some effect on the nutritive value of the diet. Chemical

analysis and feeding trials entailing several generations would have to be undertaken before any laboratory could change over to pasteurized diets with confidence.

2. Autoclaving

Autoclaving, like pasteurizing, has not been used on a large scale. Some workers have found that autoclaving did not affect the nutritive value of a compounded diet. We have found that mice grow normally on autoclaved diet 41B (Bruce and Parkes), but tests are not yet complete and we cannot confirm that reproduction is normal on an autoclaved diet which has not been supplemented with vitamins after autoclaving.

An autoclaved diet should be subjected to flowing steam for 10-15 min and then a pressure of 15 lb (120°C) for 1 h.

3. Irradiation-sterilization

Radiation has been extensively used for the sterilization of foodstuffs. The dose of radiation required will depend on the contaminants and the environmental conditions during treatment. It has been reported (Hickman, 1962) that a heavy dose of radiation causes flavour and odour changes in most foods but does not appear to affect the palatability of the food to laboratory animals. Horton and Hickey (1961) describe a technique for sterilizing guinea-pig rations by electron irradiation. This method may with modifications be used for the sterilization of other foodstuffs. Before evaluating the adequacy of a diet sterilized in this manner the authors feel further observations are necessary. Ley and Hickman (1960) found that the fat-soluble vitamins were sensitive to irradiation and reported large losses in the carotenes. The reproductive performance in rats was exceptionally poor when fed an irradiated semi-synthetic diet. The water-soluble vitamins show varying degrees of sensitivity. Proteins suffer little change in their nutritive value due to irradiation.

In 1962, after further work, Hickman reported radiation had little effect on the nutritive value of the macronutrients, and that the extent to which the vitamins were destroyed depended on the protective effect of certain foodstuffs, the temperature of irradiation and the moisture content of the feed. He agreed that the nutritive losses of irradiation-sterilization are of comparable magnitude to the losses caused by other methods of sterilization. Supplementation of the diet should ensure that the animal receives its full nutritive requirements. According to this work, irradiated diets have been fed to several species through several generations with no adverse effects on growth, reproduction, lactation, longevity or disease pattern.

4. Sterilization by ethylene oxide

The literature on the use of ethylene oxide as a sterilizing agent for foodstuffs is somewhat limited and the reports are not always consistent. It is

sufficiently well established that this method of sterilization does destroy certain essential nutrients and at the present time with such information available it is inadvisable to use it for completed diets.

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Note added in proof.

Recent work on the assessment of diets for mice is reported in *Zschr. Versuchstierk.* (1963) by Porter G., Lane-Petter, W. and Horne, N.

Chapter 3

Diseases of Laboratory Mice and Rats

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I. Introduction

A. General

There are few reasonably full accounts in English of the common diseases of laboratory rats and mice. The very exhaustive treatise by Cohrs *et al.* (1958), in German, is designed to meet the needs of experienced pathologists and is far too detailed for workers in charge of, or using, laboratory animals in general. The following chapter is therefore an attempt to give some account of the more common diseases of these two species, emphasizing those of greatest importance and dealing but briefly with those of less frequent occurrence or likely to be of specialized interest only in certain fields; for example, the pathological changes found to occur with advancing age or those found only after an exhaustive histological study has been made. A very brief section on miscellaneous diseases refers to one or two of this type.

Problems of naturally occurring diseases in laboratory animals were given little attention before about 1940. Since then, and particularly in the last 10 years, more and more attention has been focussed on such diseases of laboratory species which are potentially capable of wrecking serious experimental work. As the numbers of animals continue to increase very rapidly (in 1958 about 12,000,000 mice were used in the United States and 2,000,000 in the United Kingdom) it is obvious that the importance of this topic also continues to increase. Nevertheless, it is patent to anyone who has been intimately involved with laboratory animals over the past 30 years that the

picture has changed very considerably. The practice is growing of starting colonies with "caesarean-section-derived closed-stock",[1] in which stock are obtained by caesarean section and by fostering the young on to germ-free foster parents, or foster mothers from a colony known to be disease free. It is worth emphasizing that it is still necessary to pay attention to established principles of hygiene and husbandry. Starting with caesarean-derived stock does not necessarily preclude outbreaks of disease; such outbreaks have, in fact, been recorded. Furthermore, while such techniques can obviate disease caused by parasitic agents they cannot prevent the continued occurrence of inherited conditions, organic disturbances, neoplastic disease, diseases caused by hormone and nutritional imbalance and the so-called clinically silent lesions which occur with an increasing incidence in advancing age. Indeed, as infectious diseases are controlled, the latter group of conditions become more obvious and important.

A lifetime spent by one of us on studying disease covering a great variety of animals (including man) has provided ample evidence that the laboratory mouse suffers from a greater variety of diseases than any other laboratory species. More viral infections, latent or otherwise, are found in the mouse than in any other species. The laboratory rat is in an entirely different category —it suffers from fewer specific bacterial infections than the mouse, extremely few specific viral and protozoan infections, and spontaneous nutritional deficiencies are almost non-existent. As with all animals, the category of inherited diseases in rats is important but it is small compared with the huge number of diverse conditions seen in so many strains of inbred mice. Much of this will become apparent in the following pages.

B. CLINICAL OBSERVATIONS ON SIGNS OF DISEASE IN MICE AND RATS

To begin with it might be as well to emphasize the difference between infection and disease. An animal can be infected with a parasitic agent (whether virus, bacterium, worm or protozoon) and show no ill effects; that is, the animal suffers from a latent, occult, inapparent or clinically silent infection. More refined observations, particularly histological, bacteriological or physiological, may reveal evidence of disease, that is, structural damage to tissue, organ or system which in turn leads to functional disturbance, both or either of which can cause death.

The number of signs which can be detected by visual observation on sick laboratory animals is extremely limited, and the smaller the animal the more this is true. Nevertheless, animal technicians and those in charge of colonies ought to be taught to observe sick animals. Few diseases can be specifically diagnosed in living laboratory animals, and complicated procedures may be

[1] The term "Caesarian-section-derived" is now almost sanctified by common usage, but strictly it is incorrect, for the mother mouse is sacrified. Properly speaking, we should define this as "post-hysterectomy-delivered".

required to establish a diagnosis. Another important aspect of diseases of mice and rats is that a latent, or silent, infection may be provoked into actual disease by a large variety of factors such as irradiation, toxic chemicals, low nutritional status, changes in methods of husbandry and so on. *Corynebacterium kutcheri* infections in mice may be provoked by injections of cortisone. Cortisone injections may be necessary for the experimental transmission of *Encephalitozoön* infections in mice. *Pseudomonas pyocaneus* septicaemia can be precipitated by irradiations. In other words, if outbreaks of disease occur in stocks of laboratory animals, investigations may have to include a search for possible precipitating or predisposing factors and must not only be devoted to identification of the causal agent.

Below we list some of the clinical signs that can be noted in sick mice or rats and which should be looked for and kept in mind, but few of these signs are specific for any given disease. For example, circling, rolling and holding the head to one side may be seen very commonly in disease of the middle ear and labyrinth as well as in neurotropic infections.

1. General external appearance and conditions to be noted

Obesity, wasting, diarrhoea, appetite, urination, swellings and lumps, encrustations round external openings, sores, scabs. Condition of hair, eyes, ears, mouth, teeth, nose, external genital organs and anus. Salivation, tears, exophthalmos. Respiration may be slow, laboured, rapid or noisy. The heart may be slow or accelerated, weak or irregular. Extremities may be pale, blue or reddened or may show signs of atrophy, necrosis or oedema.

2. Neurological signs

Tremors, spasms, rigidity, convulsions, inactivity, excitability, hyperirritability, circling, holding head to one side, rolling, loss of balance, ataxia, disordered gait, loss of righting reflex, blindness, collapse, coma.

II. Bacterial Diseases

A. SALMONELLOSIS

Salmonellosis in laboratory mice is usually caused by infection with either *Salmonella typhimurium* or *Salmonella enteritidis*. Other species such as *Salm. binza*, *Salm. bredany* and *Salm. bovis-morbificans* have been isolated from time to time. Frequently these other strains do not cause serious disease and their presence may not be suspected until they are isolated fortuitously. In this chapter only bare details of the causal bacteria are given; full descriptions of the bacteria concerned can be found in standard texts on bacteriology, e.g., Wilson and Miles (1955).

Salmonellosis is one of the commonest infections of laboratory mice.

Outbreaks may occur in the form of severe, widespread epidemics or, probably more frequently, in the form of an endemic infection with low mortality and high morbidity rates. Often the latter type of infection is tolerated within a breeding colony, and the importance and scale of the infection only become evident as animals from such a colony are used experimentally, when obvious disease becomes manifest or is precipitated.

The clinical signs are as follows. The disease can run an acute, sub-acute or chronic course. In the acute form animals die less than a week after the initial infection has occurred. The signs at this stage are non-specific. Animals become less active and sit in the corner of the cage, the coat becomes ruffled and staring, and loss of weight may occur. There is rarely actual diarrhoea though faeces may become soft. In more chronic forms of the disease the clinical signs are similar but more prolonged, the animal surviving for two or more weeks.

The naked-eye findings in acute cases are those of a septicaemia. The spleen is enlarged and red, the liver slightly enlarged and congested, the intestinal mucos a reddened and the lymph nodes enlarged. Chronic cases show more specific lesions. The liver is enlarged, dark red or brownish in colour and studded with greyish-yellow focal lesions of various sizes. The spleen is considerably enlarged, dark red and congested and occasionally shows small greyish necrotic areas. The intestines are usually congested and mucosal ulcers are found. The gut contents vary from soft, almost mucoid, pale coloured material to virtually normal. In those cases with diarrhoea, congestion is particularly marked. The kidneys are rarely affected. The mesenteric lymph nodes are enlarged, congested and sometimes necrotic. Peritonitis may occur and parts of the liver, spleen and gut are covered with a thick fibrinous exudate with adhesions between the various organs. Ascites may be present. The lungs are rarely involved.

Microscopically, the findings are those of septicaemia with congestion of all organs, fatty degeneration of the liver and a marked catarrhal inflammation of the intestines. In the more chronic type of case, where the intestines are involved, there is congestion, epithelial desquamation, ulceration of the lymphoid follicles, and marked infiltration with polymorphonuclear leucocytes and histiocytes. The spleen shows congestion, degeneration of the lymphoid tissue and focal necrotic lesions. The liver shows small or large areas of focal necrosis with marginal infiltration which become confluent.

Focal necrosis of the liver in mice is not, however, specific for salmonellosis for it also occurs in Tyzzer's disease, ectromelia and a number of other ill-defined conditions. (Innes *et al.*, 1956, drew attention to the high susceptibility of the hamster to salmonella infection, and more importantly to the fact that an outstanding pathologic feature is the production of a suppurative phlebothrombosis of the lungs which is almost diagnostic. They indicated that the same type of lesion can occur in experimental mice but to a lesser degree in the lungs, although the literature on salmonellosis does not indicate that this

highly significant feature has ever had any attention paid to it. A recent account of airborne *Salm. typhimurium* infection in mice by Darlow *et al.*, 1961, is of considerable interest in this respect.)

Carriers (i.e., infected, but not sick, individuals) capable of excreting *Salmonella* organisms intermittently over a long period are very frequently found among the survivors of epizootics, and these mice constitute the greatest obstacle to ensuring the elimination of the pathogens from stocks once they are infected. These animals can only be detected with assurance by bacteriological examination after death—even prolonged serial faecal cultures cannot be relied upon to detect all carriers. Sero-diagnosis of infected individuals is likewise unreliable (Sacquet, 1959). Various stimuli can lead to outbreaks, particularly overcrowding, infrequent cage-cleaning and sterilization, the use of food and bedding contaminated by wild rodents, and inadequate quarantine precautions for new stock. Nutrition can influence the outcome of *Salmonella* infections (see the work of Schneider, reviewed in Schneider, 1962) and a variety of experimental procedures may light up inapparent infections. Salmonellosis may sometimes be precipitated with drugs in the course of routine acute or chronic toxicity tests.

Diagnosis depends upon the isolation of the causal organisms. The finding of suspicious lesions is a valuable guide, but these are not always sufficiently distinctive to ensure an accurate diagnosis. *Salmonellae* may be isolated by the standard bacteriological techniques, employing selective media such as desoxycholate-citrate agar, Wilson and Blair's medium, and Selenite-F or tetrathionate enrichment broths. In practice, liver, spleen and rectal contents are the most useful sources to culture, but *Salmonellae* can also be sought in heart-blood, mesenteric lymph nodes, peritoneal exudate and gall-bladder. Following the recovery of suspicious non-lactose fermenting colonies, slide-agglutination methods can be used to make a rapid identification. The organism may be fully typed by means of the usual serological techniques, but there is little point in doing this unless one is particularly interested in the epidemiology of outbreaks.

Satisfactory control of this disease, i.e., complete elimination of the organism from a mouse stock, is extremely difficult, but this organism is such an important parasite of mice that nothing short of complete elimination should be accepted as satisfactory. Chemotherapy and vaccination measures are useless. The only really satisfactory control procedure is to destroy the stock completely and obtain fresh, tested, known *Salmonella*-free stock. Where a mouse strain must be preserved, one can attempt to re-found the stock from selected breeders which have been thoroughly tested for the absence of *Salmonella* organisms by bacteriological techniques. If this latter method is resorted to, it is important that new foundation breeders should be kept in rigid isolation with absolutely no chance of contact, direct or indirect, with any previously infected animals. Furthermore, it must be remembered that

even the excretion of fourteen daily faecal samples free from *Salmonella* does not necessarily preclude infection of the animals, as demonstrated by the culture of various tissues taken at autopsy (Hammond and Bohnoff, 1954).

In rats, salmonellosis has never been an important problem to breeders of large colonies. Some reference to the disease appears in the older literature. The disease occurs occasionally in the wild rat. However, in the experience of one of us going back 30 years and covering tens of thousands of rats, no outbreak has ever been seen, nor is there remembrance of ever meeting anyone who has seen the disease in rats.

According to literature (see Cohrs *et al.*, 1958, for references), the disease in rats resembles in general that seen in the mouse, except that serofibrinous pleurisy, peritonitis and bronchopneumonia have been recorded as well as the multiple focal necroses seen in liver and spleen. Buchbinder *et al.* (1935) give a very full account of an endemic, chronic infection in a rat colony.

B. TYZZER'S DISEASE

The disease was first described in Japanese waltzing mice by Tyzzer (1917) who ascribed the infection to an organism which he termed *Bacillus piliformis*. Subsequent observations by Tuffery and other workers would suggest that the causative agent is unlikely to be a member of the *Bacillaceae*, yet the term is now in common usage and pending adequate systematic studies of this agent the term will be used here for convenience.

The disease is one of the major causes of losses among laboratory mice in Europe and Japan. Since the original description, outbreaks have been reported from laboratories all over the world, yet there have been few adequate accounts of this disease published. No other laboratory animals are susceptible naturally, although encephalitis can be produced in rats, rabbits and hamsters by intracerebral injection of the organisms.

The clinical signs are as follows. Tyzzer's disease is a hepatitis with intestinal involvement. In epidemic outbreaks the acute form is that most often seen. Animals appearing to be in perfect health one day are dead the next. Slightly more chronic cases may show loss of weight and a general deterioration in condition, with the usual staring coat, and humped-back, listless appearance of the sick mouse. There is often a diarrhoea of varying severity, with death following fairly rapidly in young mice, and between 2 and 3 weeks later in mice 5 weeks old or more. Chronic cases show prolonged loss of condition but very often remain quite undetected or unsuspected until autopsy.

Little or nothing is known of the factors which predispose towards the outbreak of an epidemic of Tyzzer's disease, but the experience of Gard (1944) suggests that unhygienic methods of offering food to mice might tend to promote an epidemic. Hadlow showed that cases can occur if mice are kept on the same bedding for prolonged periods. Recovered animals are said to possess immunity.

Diagnosis of this disease rests upon the finding of the typical macroscopic, target-shaped, focal necrotic lesions in the liver and the histological demonstration of peripherally infiltrated necrotic areas surrounded by parenchymal cells in which *Bacillus piliformis* can be found.

Outbreaks of Tyzzer's disease are difficult to control because the disease as a whole is so little understood. In practice, however, it is quite possible to keep a stock of animals free from infection by means of normal hygienic measures and rigid restriction of the introduction of the stock from other colonies. Should outbreaks occur, the only reasonable control methods known at present consist of culling all sick mice and contacts of sick mice with the typical necrotic liver lesions at autopsy.

C. *Corynebacterium kutcheri* INFECTION

Infection with *Corynebacterium kutcheri* (*C. pseudotuberculosis murium*) is mainly an inapparent one in laboratory mice under normal conditions. In large colonies, small numbers of mice may be found with liver lesions, but these are rare.

Clinical signs of infection with this organism are rare and non-specific. There may be a loss of weight and condition, but often this is not very marked. Rapidly fatal cases seem to be uncommon. The most frequent pathologic finding is that of pin-head, or sometimes larger, necrotic spots in the liver which later become small abscesses. These spots may protrude from the surface of the liver forming small nodules. Less frequently in naturally occurring cases large caseous areas, in which bacilli are abundant, are found in the lungs. Rarely, the lymph nodes of the axilla, neck, mediastinum and mesentery may become enlarged and abscessed. More extensive lesions may be found in cases produced in the course of experimental work for some other purpose.

Both naturally occurring, and experimentally induced, cases of this disease have been called pseudotuberculosis.[1] The liver, lung and lymph node lesions are abscesses containing numerous bacilli. The organism is apparently toxigenic. *C. kutcheri* is a typical member of the *Corynebacteriaceae*; it is gram-positive and somewhat pleomorphic, with irregular and club-shaped forms which frequently stain in a granular or segmented manner. It is readily isolated with standard laboratory media. Texts on bacteriology provide data to permit of its identification.

Infection with this organism is likely to be inapparent until the animals are subjected to some form of experimental stress. Infection with *Ectromelia* or *Salmonella*, poor nutrition, injections of cortisone and irradiation can all change clinically silent cases to clinically apparent and sometimes very severe

[1] The term pseudotuberculosis probably should be discarded for it bears no resemblance to tuberculosis other than in the occurrence of caseous lesions of lymph nodes. Further, the term has also been applied to a *Pasteurella* infection in guinea-pigs and rats.

cases. For this reason the organism is not likely to cause many deaths or much inconvenience in breeding colonies but can be a considerable hindrance to, or invalidate, experimental work. The use of some stressful procedure is an obvious means of screening a breeding stock for infection. The experimentally induced disease may have a very high incidence rate. Infection occurs in rats, but in these it is always inapparent in normal animals.

Diagnosis depends on the isolation of *C. kutcheri* from liver, lung and other lesions.

Epidemics among normal laboratory mice are very rare and in such outbreaks control can be obtained by culling sick individuals and their cage mates. Where the infection rate is high it may prove necessary to consider re-founding the stock from offspring of parents shown to be free from infection after irradiation or after cortisone inoculations.

D. *Streptobacillus moniliformis* INFECTION

Before describing this infection, and mouse catarrh which follows, a few remarks concerning *Streptobacillus moniliformis* and the pleuropneumonia-like organisms (PPLO *Mycoplasma*,) are required, since the earlier accounts of these organisms and their related diseases are confusing. *S. moniliformis* spontaneously gives rise in culture to variants termed L-forms. These L-forms are morphologically very similar to the PPLO and it is still not possible to decide upon the interrelationships of these organisms. (See Klieneberger-Nobel, 1962, for a full account.) Furthermore, non-pathogenic variants (or close relatives) of the pathogenic forms exist, frequently in animal tissues, and their isolation from time to time has led to confusion as to the role members of these groups play in the conditions observed.

Disease due to *S. moniliformis* is not very common among laboratory mice although it is probably a fairly common parasite of this species. *S. moniliformis* is a gram-negative, extremely pleomorphic organism on first isolation, but it tends to take a more typical bacillary form in old laboratory cultures. For a description of the organism see Wilson and Miles (1955) and Freundt (1956).

The disease in mice usually takes a moderately chronic form, but outbreaks with many acute cases have been described. The outstanding clinical sign is marked oedema of feet and legs, and arthritis. Gangrene or amputation of the foot is not common, but the lesions must be distinguished from those caused by the virus of *Ectromelia* (mouse pox). Conjunctivitis is frequent and often there is cyanosis of the extremities. Swellings are often seen on the tail and submaxillary abscesses may form. Freundt (1956) found diarrhoea in some mice. The acute form is a severe systemic infection and is rapidly fatal.

At autopsy, apart from the arthritis (see later remarks) the only other regularly occurring lesions consist of areas of focal necrosis in the spleen which in severe cases become confluent. The liver is less often involved. The intestines may be congested.

Experimentally the disease can be readily reproduced by intraperitoneal, intravenous or subcutaneous inoculations of fresh isolates of the causative organism. Stock cultures of this bacterium appear to lose their virulence rather rapidly.

There is suggestive evidence that rats kept in the same room as a stock of mice can act as a reservoir of *Streptobacillus* organisms from which the mice become infected. There is one account of mice being directly infected as a result of inoculation with rat-derived material. As already explained, outbreaks are unlikely, but the best guarantee of freedom from infection probably lies in the use of techniques, as described in Chapter 4, designed to establish disease-free colonies.

In rats, *S. moniliformis* has been recovered many times from purulent infections of the middle ear, and from the large nodular caseous lesions present in the late bronchiectatic stages of chronic murine pneumonia. While reports on the presence of a natural joint disease as described in mice have not been so frequently reported in rats, there are a number of studies on experimental production of bone and joint infection of rats by the same organism. A recent one by Lerner and Sokoloff (1959) is important in that direction, and most of the other references on this topic can be obtained from their paper. These workers (and many others quoted by them) showed that a strain of *S. moniliformis* recovered from a middle-ear infection in a rat was capable of producing inflammation of bones and periarticular tissues within 24 h after injection. Swelling and reddening of joints pointed to involvement of the extra-articular tissues, but it is bone which is the primary seat of involvement and not synovial tissues. The lesion is in effect an infectious osteo-arthritis but starting as an osteomyelitis.

Comments on natural degenerative joint diseases of small laboratory animals appear at the end of this chapter, and should be read in conjunction with what has appeared on the infectious types.

E. INFECTIOUS CATARRH

Infectious catarrh is a chronic disease caused by pleuropneumonia-like organisms (PPLO, *Mycoplasma*). It is very widespread, being found in most mouse and rat colonies not founded by techniques specifically designed to eliminate it. It leads to a general lowering of standards of health, with a low mortality but very high infection rate which only may become evident in mice and rats when they are examined at the end of some chronic experiment. Some 20 years ago, before the work of Nelson, this infection was a great stumbling block towards the solution of other pathologic entities in both species.

The clinical signs are as follows. In mice the most characteristic symptom of this disease is a chattering or clicking noise, readily heard in a quiet animal room. As the disease progresses, particularly as pneumonia supervenes, the animal becomes less and less active, the coat stares and the animal becomes

more and more sick. Rhinitis, although usually present, never shows externally—there is no accumulation of secretions round the nostrils. Otitis media is usually detected at autopsy but occasionally the labyrinth is involved, and such animals hold their heads in the typical lopsided position indicative of interference with the organs of balance. Other mice and rats will indulge in constant circling or rolling, partly due to involvement of the middle ear, but also with labyrinth infection. There may be extension of the inflammatory process through to meninges and brain. Rats do not chatter, but tend to snuffle, and there may be blood-stained encrustations round the nose.

The lesions are confined to the nasal passages, middle ear and lungs. Commonly, rhinitis is indicated by the presence of profuse semi-fluid exudate in the nasal passages, but sometimes there is little exudate and it is necessary to examine Giemsa-stained films prepared from washings of the nasal cavities. Inflammation is indicated by the presence of polymorphonuclear leucocytes and desquamated epithelial cells. Occasionally it is also possible to demonstrate PPLO (Nelson's "cocco-baccilliform" bodies) in or adjacent to cells in stained films. Otitis media is revealed by an obvious exudate in the middle-ear cavity or by a less obvious exudate which again requires microscopic demonstration by films. Infection may be unilateral or bilateral. Less frequently there is lung involvement—a slow, progressing bronchopneumonia with mucoid exudate plugging bronchi. Often there is a marked peribronchial lymphoid hyperplasia.

It is difficult to be certain how the middle ear is infected. Infection can spread from an abscess at the back of the throat and nasopharynx through the bone into the middle and inner ear. A transverse section of the whole head through the middle ears is a good way of demonstrating the presence of lesions and infection.

Lung lesions are fairly frequent, but in such cases the chances are the animals may have had dual infection, by the PPLO on top of the virus of chronic murine pneumonia which is discussed later, for in pure PPLO infections chronic pulmonary lesions do not occur (see in this connection Pankevicius, *et al.*, 1957).

Nelson, in a long series of papers, has thoroughly investigated this problem and a similar condition appearing in rats (see below) (summarized in Nelson, 1958, 1962). It can be reproduced in uninfected mice by the intranasal inoculation of cultures of PPLO or exudates. Inoculated animals start chattering in 10 to 14 days. After 4 weeks rhinitis, otitis media and pneumonia in varying combinations can be found at autopsy. Intraperitoneal, intravenous and footpad inoculation of certain strains of PPLO can give rise to an arthritis. In female mice intraperitoneal inoculation is often followed by inflammation of the Fallopian tubes and ovarian capsule (Nelson, 1958).

Diagnosis of the disease depends upon the finding of rhinitis and otitis media in chattering mice and snuffling rats. Usually film preparations of the

nose and middle ear are sufficient to establish the diagnosis, but pleuropneumonia-like organisms can be readily cultured on Klieneberger-Nobel's medium (1962) or Nelson's serum medium. An enrichment culture in liquid medium (used by one of us, A. A. T.) before sub-culturing on to a solid medium for identification has led to a higher percentage of recoveries.

Once this infection is widespread in a stock it is difficult to eradicate. Culling of chattering mice and snuffling rats appears to help, but usually the agent is too widespread for this to be very effective. The only real control is to resort to one or other of the techniques described in Chapter 4 or by fostering new-born animals on to adult females from a known uninfected stock.

F. OTHER PPLO MYCOPLASMA INFECTIONS

Pleuropneumonia-like organisms can also give rise to a low-grade conjunctivitis in mice (Nelson, 1950). Various organic gold and arsenic compounds appear to have a useful therapeutic effect on these infections.

Further valuable descriptions of infection due to PPLO are given by Dingle (1941) and Edward (1947). See also Wilson and Miles (1955).

G. STREPTOCOCCAL INFECTIONS

Streptococcal infections among mice and rats are rare but two accounts are worth mentioning, one due to a group A, type 50, *Streptococcus pyogenes*, the other due to an enterococcus.

Hook *et al.* (1960) describe an outbreak of group A, type 50, streptococcal adenitis, in their Swiss mice, and also refer to outbreaks in three other strains. This outbreak was presented as a cervical adenitis with gross enlargement of the submaxillary lymph nodes and the formation of abscesses which subsequently regressed. Mortality rates reached 50%, with deaths 3 to 4 weeks after infection due to systemic spread of the organisms, with bacteraemia, pneumonia, and very occasionally meningitis. The mice (which were bought from a commercial breeder) proved to be chronic carriers with organisms readily isolated from their throats. The carriers infected normal animals. A similar outbreak due to the same type of streptococcus is described by Nelson (1954).

Gledhill and Rees (1952) describe an outbreak due to a group D enterococcus. The organism was first detected in cortisone-treated mice, but subsequently untreated animals began to show signs of illness when the colony was subjected to overcrowding and poor temperature control. Death followed within 48 h of the first signs of ill-health. Occasionally there was a diarrhoea. At autopsy the liver was always affected, showing small irregular whitish areas which subsequently enlarged, coalesced, and became very extensive. In very acute cases the lesions were less developed. The alimentary canal showed numbers of pin-head sized whitish spots surrounded by halos

of inflammation. Histologically the liver lesions were irregular and focal in character, consisting of degenerate, necrotic liver cells surrounded by narrow zones of cell infiltration. Enterococci could be seen in these lesions.

Streptococcal infections are rare among mice and rats, but should outbreaks occur, vigorous culling measures should be taken, with the possibility of carriers occurring kept in mind.

H. PASTEURELLOSIS

Infection with *Pasteurella pseudotuberculosis* is rare among mice and rats although it is common in other laboratory animals, especially guinea-pigs and rabbits. Sellers *et al.* (1961) describe the recovery of great numbers of *Past. pseudotuberculosis* organisms from the lungs of mice infected 5 to 10 weeks previously with influenza virus. The presence of this intercurrent infection considerably altered the histological picture of influenza infection in these animals—they developed a purulent bronchopneumonia instead of the uncomplicated bronchopneumonia which develops with influenza virus alone.

Acute pneumonia in laboratory rats, caused by any variety of microbial agents, is a relatively rare event, certainly in well-run colonies even if these are not completely isolated. One of us (J. R. M. I.) did not see a single epizootic for some 25 years, then saw and heard of four outbreaks at the same time, in different areas, which killed many animals. Death was rapid and in each case the essential feature of the disease (pathologically) was an acute suppurative pneumonia spreading by bronchial pathways—essentially a bronchopneumonia. Some animals also showed myocarditis and hepatitis. *Pasteurellae* were recovered, but it was extremely difficult to reproduce the disease in all its ramifications.

I. SPIROCHAETOSIS

Spirochaetal infections of wild, not laboratory, rats have been known for a number of years, but infections with *Leptospira icterohaemorrhagiae* and *Spirillum minus* have been recorded but rarely in laboratory mice. Such infections as occur do not lead to overt disease. Of more importance is the possible risk to animal technicians of contracting rat-bite fever (or more properly mouse-bite fever) from infected animals.

A less likely risk is that confusion might arise as to the meaning of tests involving the injection of material into mice for the diagnosis of spirochaetal infections.

Dingle (1941) gives a brief summary of these infections, with references prior to 1941. Stoenner *et al.* (1958) gave an account of *Leptospira ballum* infection and control in mice.

J. *Pseudomonas pyocyaneus* AND MISCELLANEOUS INFECTIONS

Pseudomonas pyocyaneus (*Ps. aeruginosa*) is occasionally recovered from subcutaneous abscesses and the middle ears of mice showing symptoms of

labyrinthitis. Other organisms, such as *Staphylococci*, *Streptobacillus moniliformis* and PPLO may also occur in such situations.

Ps. pyocyaneus, *Proteus* spp, *Bact. coli* and other coliform organisms can be troublesome parasites in animals used for irradiation experiments. Very often mice die 3 or 4 days after receiving heavy doses of irradiation, and autopsy reveals a bacteraemia due to one or other of these organisms, especially *Ps. pyocyaneus*.

It is usually considered that such organisms derive from the gastro-intestinal tract of these animals, but Wensinck (1961) has recently produced evidence to show that such organisms may originate from the respiratory tract. Unless animals are used for radiation work, these organisms may be tolerated as unimportant parasites, but for certain projects it may be necessary to eliminate mice carrying such organisms.

Haemophilus bronchisepticus and *Klebsiella pneumoniae* (Friedlander's bacillus) have both been implicated in outbreaks of epidemic pneumonia among mice, but such occurrences are rare. Naturally occurring pneumococcal pneumonia has been reported in rats but not in mice.

III. Viral Diseases

A. General

There is a great variety of spontaneous viral infections known to infect laboratory mice, but most of these are almost completely inapparent or latent in normal animals. These infections have little effect upon their hosts (so far as is known at present) under a variety of experimental conditions. Their presence is usually only suspected when unexpected results are obtained during the course of certain laboratory procedures, of which the most important are serial passage (mouse to mouse) of tissue suspensions, e.g., by intranasal inoculations, by passage of tumours, by using tissue-culture methods with cells of murine origin, by inoculation of mice (especially very young mice) with material originating from other species (for diagnostic purposes, e.g., lymphocytic chhoriomeningitis), and serological tests designed specifically to detect these infections. Of these, serial passage of tissue suspensions and tumours or tumour cells are undoubtedly the most important.

Table I summarizes the most important characteristics of 15 latent murine virus infections. Most of these will be of little interest to many users of laboratory mice, but they could well be very important to workers in certain fields of virology and cancer studies. Reference should be made to the original papers for full details. Of those listed, PVM, Nigg's pneumonitis and MHV have been most thoroughly investigated—information on many of the others is still fragmentary. Several important viral diseases are dealt with separately. An important recent paper by Rowe *et al.* (1962) gives a valuable account of the incidence of a number of latent mouse viruses in mouse stocks.

In rats, the problem is relatively simple, for we need only deal with the

TABLE I

Characteristics of some Latent Viral Infections of the Mouse

Name	Major site of infection	Natural disease manifestations	Experimental disease manifestations Signs	Pathology	Mode of discovery	Diagnosis by means of:	Relationships	Chemotherapy	Infectivity for other species	References
PVM (pneumonia virus of mice)	lungs	—	non-specific illness	consolidation of lungs	serial passage of lung suspensions	inoculation CFT HA and A/HA neutralizing tests		—	—	Mills and Dochez, 1945 Horsfall and Hahn, 1940
Mouse pneumonitis (Nigg pneumonitis)	,,	—	non-specific illness; rapid death	pulmonary congestion or focal, greyish, pin-point lesions. Elementary bodies.	,,	neutralizing tests; chick embryo culture	serologically distinct	aureomycin	hamster	Nigg and Eaton, 1944
GLV (Grey lung virus)	,,	minimal lung lesions occasionally seen	non-specific illness	greyish-red pulm. congestion and oedema; later emphysema, bronchiectasis	,,	inoculation	? virus	aureomycin terramycin arsenicals	rat, hamster, vole, guinea-pig	Andrewes and Glover, 1946
Agent of Dochez *et al.*	,,	—	rapid death	pneumonia	rapid serial passage	poorly antigenic	related to Gordon *et al.*?	—	ferrets	Dochez *et al.*, 1937
Agent of Gordon *et al.*	,,	—	,,	,,	,,	,,	related to Dochez *et al.*?	—	?	Gordon *et al.*, 1939
K virus	,,	—	adults—nil; 10 days old—laboured breathing, rapid death	adults—nil; 10 days old—gross lung congestion	inoculation of very young mice with various mouse tissue suspensions	inoculation of young mice, histology, neutralization tests	serologically distinct	—	—	Kilham and Murphy, 1953
Karr	,,	—	non-specific illness; death in 3 days	pin-point grey foci to greyish pink consolidation	serial passage of lung suspension	neutralization tests	? Dochez *et al.* (1937) Serologically distinct from PVM	—	hamsters	Karr, 1943
Mouse adenovirus		—	death 5-7 days post inoculation in sucking mice	lesions in various organs	cytopathic effects in tissue cultures	CFT, neutralization tests	an adenovirus	—	—	Hartley and Rowe, 1960
MHV (Mouse hepatitis virus)	liver	minimal lesions in liver	non-specific illness; some strains neurotropic	focal liver necrosis	passage of liver suspensions in *E. coccoides* infected mice	inoculation with *E. coccoides*	similar to Nelson hepatitis virus	arsenicals, aureomycin, and terramycin will control *E. coccoides*	—	Gledhill and Andrewes, 1951 Gledhill *et al.*, 1955 Nelson, 1952; 1955
JHM Agent of Morris and Aulisio	,,	,,	flaccid paralysis	encephalomyelitis and liver necrosis	serial passage	neutralization tests inoculation	related to MHV	—	—	Cheever *et al.*, 1949 Morris and Aulisio, 1954
Agent of Braunsteiner and Friend	,,	—	loss of weight and non-specific illness	focal necrosis of liver	liver lesions after urethane treatment	inoculation of urethane-treated mice	?	—	—	Braunsteiner and Friend, 1954
AHA (ascites hepatitis agent)	,,	—	ascites, low mortality	hepatosplenomegaly and ascites	serial passage	—	differs from MHV; possibly related to Lackey *et al.*	—	—	Jordan and Mirick, 1955 Lacky *et al.*, 1953
HEV (hepato-encephalomyelitis virus)	,,	—	very pathogenic for sucking mice	hepatic and neural lesions	? mouse origin	—	differs from JHM/MHV group	—	—	Stanley *et al.*, 1954
Inclusion bodies in liver cells	,,	—	—	—	during histological studies	histologic examinations	?	—	—	Campbell, 1939; Findlay, 1932; Thompson, 1936 Pavilanis and Lepine, 1949
EMC (Encephalo-myocarditis virus)	CNS	—	paralysis	myelitis, myocarditis	inoculation of chimpanzee material	—	related to Columbia, SK, MM, and Mengo-encephalomyelitis	—	—	Warren *et al.*, 1944; Committee on Nomenclature, 1948
Salivary-gland virus	salivary gland	—	death 3-7 days post inoculation	death 3-7 days post inoculation	histology; cytopathic effects in tissue cultures	neutralizing tests, histology tissue, culture	?	—	—	Mannini and Medearis, 1961 (gives earlier refs.)

complex of chronic murine pneumonia and salivary gland virus infection. The viruses which produce neoplastic diseases are dealt with under that heading.

B. MOUSE POX (INFECTIOUS ECTROMELIA)

Mouse pox is caused by the murine representative of the pox group of viruses, *Poxvirus muris*. This disease is one of the most important intercurrent infections in laboratory mouse colonies, outbreaks having been reported from many European countries and from America. Colony infection can take the form of violent epidemic outbreaks or low-grade endemic conditions, or often remain totally undetected until animals are subjected to some form of experimental stress.

The original description by Marchal (1930) gives an excellent account of the disease. Fenner (1949) has studied the pathogenesis and epidemiology of this disease extensively, and Briody (1959) has a valuable, more recent, review of the subject.

The clinical signs are as follows. Acute and chronic cases occur. Clinical signs in acute cases are non-specific. The animal becomes sick, lethargic, anorexic, emaciated, and develops a staring coat. The disease progresses rapidly and death may occur in 24 h. Pathognomonic lesions are seen in more chronic cases. Infection is first indicated clinically by the presence of a primary lesion which usually takes the form of a swelling on the face. The disease may progress rapidly from this stage and the animal may die with no more obvious clinical signs, or a generalized skin rash may develop over most of the body. This secondary rash consists of dry, scabby, sometimes very extensive, lesions, which are particularly evident on the feet and tail but which can be seen on the hair-covered parts of the body as well. Occasionally the foot lesions progress and the whole foot becomes necrotic and eventually sloughs off. Amputation is not an invariable or even frequent occurrence, as Marchal suggested. Conjunctivitis occurs very frequently.

Acute cases show few specific lesions. There may be some congestion of the liver and excess peritoneal fluid, but little else. More chronic cases show rather more typical lesions. The liver may be pale, friable and covered with greyish-white areas of focal necrosis. The spleen may be somewhat enlarged and similarly show necrotic areas. There may be some peritoneal exudate and often the contents of the small intestine in the duodenal region are blood-stained (this last lesion is sometimes lacking). Other organs in the abdomen and thorax are rarely involved.

Histologically, the liver in chronic cases contains numerous focal necrotic areas which may, in severe cases, coalesce. These lesions show no inflammatory infiltration. Necrosis of the spleen can also be very extensive, and in mice which have recovered from severe chronic infections such necrosed areas are replaced by fibrous tissue, a feature Fenner regards as pathognomonic of

this disease. Inclusion bodies can be demonstrated fairly readily in the epidermal cells of the skin in areas of inflammation. Inclusion bodies can also be found in the acinar cells of the pancreas, and in the liver (but in the latter they are more difficult to demonstrate), which serves to differentiate other diseases with hepatic necrosis (e.g., salmonellosis).

The chronic disease with skin and foot involvement can be readily induced experimentally by inoculation of liver or spleen suspension containing virus into the foot-pad of normal mice. Such animals show signs of sickness at about 7 days after inoculation and by 13 or 14 days the typical skin rash has developed. Intraperitoneal inoculations of virus-containing material may result in acute or chronic disease, depending upon the dosage and virulence of the virus strains.

Outbreaks of the infection are likely to occur when fresh stock from other colonies are introduced into an animal house already containing a stock of infected mice. Outbreaks are also likely to be precipitated or detected among animals in use in the laboratory. The virus is often discovered during procedures involving tissue passage in mice, especially of tumours.

Ectromelia is usually first suspected when mice bearing fairly extensive skin lesions are discovered. Earlier clinical signs than this are often missed. Diagnosis depends upon six factors. 1. The finding of typical lesions on the face and eyes, feet, tail and other parts of the body. 2. The production of a similar disease by foot-pad inoculations of liver suspension prepared from sick animals. 3. The isolation of a pock-producing virus from similar liver suspensions on the chorio-allantoic membranes of ten- or eleven-day old chick embryos. 4. The demonstration of the presence of haemagglutination-inhibition antibodies in the serum of samples of mice taken from a colony. 5. The presence in a colony of a high proportion of non-reactors among mice which have been vaccinated by scarification of the skin of the base of the tail with sheep-lymph vaccine. 6. The demonstration of inclusion bodies in epidermal (and other tissue) cells at the appropriate stage in the disease.

In practice, the finding of suspicious skin lesions, the demonstration of a disease-producing agent in bacteria-free liver suspension, and the use of the haemagglutination-inhibition test described by Briody (1959) will establish the diagnosis sufficiently firmly for action to be taken in the event of an outbreak.

Where stocks of animals are bought at intervals they should be quarantined for a period of 2 to 3 weeks before they are allowed to be introduced in the normal animal rooms. During this period they should be carefully observed for signs of disease. A further very useful safeguard, where animals are used for long-term experiments, is to vaccinate such animals before use (see Salaman and Tomlinson, 1957). Should a severe epidemic outbreak occur in a breeding colony, undoubtedly the safest procedure is to eliminate the entire

stock, disinfect all the equipment and rooms which have been in contact with these animals and re-found the stock from known ectromelia-free sources. Where it is important to keep a strain, or where for other reasons it is not desired to kill off large numbers of animals, the procedure described by Tuffery (1958) may be used. In essence, this method consists of selecting small numbers of apparently healthy mice, confining them under conditions of rigid isolation, vaccinating all these animals, rejecting any sick animals which may develop, and rebuilding the stock from such animals in complete isolation. This stock can then be used to replace the infected stock.

C. INFANTILE DIARRHOEA IN MICE AND RATS

Widespread epidemics, with high morbidity and continuous high mortality rates, have been reported from many laboratories in Europe and America. Aetiologically, two distinct viruses seem to be involved (Kraft, 1962).

Since the work of Cheever and Pappenheimer it has become obvious that there may well be more than one form of infantile diarrhoea in sucking mice. Indeed, Pappenheimer came to that conclusion, largely on the basis of finding intranuclear inclusion bodies in intestinal epithelium in one outbreak and intracytoplasmic ones in another. Previously, in another outbreak, *Salmonella* was recovered from infected animals. One of us (J. R. M. I.) has also seen an outbreak which started explosively and terminated in 2 weeks just as suddenly, and in which no inclusion bodies of any variety were found.

It is not generally appreciated that an apparently similar condition occurs in the laboratory rat, and the only report is that by Foster (1958). The clinical signs were approximately the same, but mortality was very low. Histological examination of the intestines did not reveal any entero-colitis, and the presence of inclusion bodies was not proved.

The clinical signs are as follows. The disease is usually first noticed by those in charge of an animal colony when numbers of baby mice are found to be unthrifty and excreting a profuse yellowish diarrhoea which, in the worst cases, comes to cover the coats of entire litters as the mice climb over each other. Less clinically obvious cases may be missed. Often young mice develop a diarrhoea which is not detected at once because the mother mouse is able to keep the animals clean by grooming them. At a later stage, the discharge becomes too profuse for her to cope with and the disease is then noticed for the first time. Diarrhoea usually occurs at 10 to 12 days of age. Prior to the appearance of disease among the young, the mother's coat shows a curious moist or damp appearance. There is often a tendency for first litters, but not subsequent litters, to be affected, but in some outbreaks (particularly in colonies with a long continued history of endemic infantile diarrhoea) this tendency becomes less marked. Litters born subsequent to infected litters are frequently not affected, or, if affected, suffer a milder attack. Once diarrhoea becomes obvious it proceeds for 2 or 3 days, and then either the

animals recover, or it progresses, the discharge becoming dryer and the animals eventually die within a further 2 or 3 days from gross impaction of the anus with a solid mass of black matter. Animals that recover usually show marked stunting and retardation of growth.

Females with an infected litter show no macroscopic or microscopic changes. Young, unweaned mice killed at the height of the disease may have a fluid, greyish to bright yellow mucoid material in the lower region of the colon and the rectum but virtually no other abnormal changes.

The histological appearance of gut sections from cases of infantile diarrhoea has been described by Cheever and Mueller (1947, 1948), Pappenheimer and Cheever (1948) and more recently by Kraft (1962). Sections of various regions of the intestines of young mice killed at the height of the disease show no inflammatory reaction of any kind, but two types of inclusions are found in the columnar epithelial cells lining the villi of the small intestine. These inclusions appear to be difficult to demonstrate. Kraft, in her more recent work, described different types of inclusions. It must be concluded that the aetiology of this disease, whilst it is probably viral, is likely to be different in separate outbreaks.

The disease is difficult to reproduce experimentally unless the special isolation techniques developed by Kraft are employed.

Factors which influence the outbreak of epidemics are quite unknown, although overcrowding, a general low standard of hygiene, and making the females breed hard might well predispose towards outbreaks. Seasonal variations can sometimes be demonstrated.

Diagnosis rests upon the prompt recognition of typical clinical signs as described above, and the autopsy appearances of the large gut in young mice 10 to 14 days old. Control of this infection is very difficult. In fresh outbreaks with low incidence rates, culling affected litter, and their parents will probably keep the outbreak within limits, but it is very difficult to prevent the disease spreading throughout the entire stock. The only sure control can be obtained by the use of caesarean and fostering techniques, or the selection and careful observation of supposedly uninfected pregnant females and their isolation in filter cages as described by Kraft. Where a breeding nucleus of disease-free stock has been founded it can be expanded to replace the infected colony, but rigid isolation precautions must be employed during this process.

D. LYMPHOCYTIC CHORIOMENINGITIS (LCM)

Lymphocytic choriomeningitis is a widespread viral infection of laboratory mice, which in its rare, but most acute, form produces a rapidly fatal paralysing meningoencephalitis. A large percentage of house mice are infected and it has occurred as a dual infection with distemper in dogs. The virus has been isolated from ticks. Most mice, however, carry the virus in latent form. It is a hazard to human health, since laboratory workers have become infected from

handling mice, and it is likely to invalidate experimental work with other neurotropic viruses. Animal workers should remember that the virus can penetrate the intact skin, and that the urine of infected mice contains virus. Few colonies are free of the disease unless they have been founded by techniques specifically designed to exclude it. Apart from mice, the virus has been isolated from monkeys, dogs, guinea-pigs and man. Experimental infection can be induced in a wide range of animal species.

In spite of the widespread nature of the infection, clinical disease is rare. Under endemic conditions, infection occurs *in utero* or very shortly after birth. Virus is excreted by infected animals in the nasal secretions and urine, and some infected mice remain carriers for months and act as sources of virus for new-born or other uninfected animals. Contact infection is probably acquired *via* the respiratory route, and these cases show few or no signs of disease. Those infected *in utero* may show a decreased growth rate, emaciation, slow stiff movements, slight somnolence, ruffled fur, and occasionally diarrhoea about a week after birth.

Only minimal lesions are found at autopsy—pleural exudate, fatty liver and enlarged spleen being found occasionally. Microscopically, there is a leucocytic infiltration of the meninges but this is never very extensive. Peribronchiolar and perivascular infiltrations of round cells have been described in the lung, and small collections of round cells may be seen in the liver. The lesions, both microscopically and macroscopically, are not very remarkable.

The disease can be reproduced by intracerebral, intravenous, subcutaneous and intraperitoneal inoculations of virus-containing suspensions of brain or other materials from infected animals. Intracerebral inoculation in animals free from intercurrent LCM infection results in a general malaise by about the 6th or 7th day and these animals may convulse when picked up by the tail. Subcutaneous and intranasal inoculation of infected brain suspension results, at the most, in a mild form of the disease. Intraperitoneal inoculation results in a severe, but not always fatal, infection. Microscopically, there are mononuclear infiltrations mainly at the base of the brain, in the meninges and chorioid plexuses.

Diagnosis of LCM infection must rest upon (i) the isolation of virus from infected mice, and cross immunity tests with a known strain of the virus; (ii) the demonstration of complement fixing antibodies in mouse serum; and (iii) the stimulation of inapparent to overt infection by intracerebral inoculations of sterile broth or starch suspension. Material from mice suspected of containing LCM virus can be inoculated intracerebrally into known uninfected mice or guinea-pigs. Intracerebral inoculation in the guinea-pig results in fever and death within 9 to 16 days.

Control of lymphocytic choriomeningitis in a mouse colony must depend on the foundation of a breeding unit from a small number of mice which have been demonstrated to be free of the infection by the appropriate tests.

The simplest of such tests is probably challenge by the intracerebral route with known LCM virus. Animals carrying an inapparent infection will survive such a challenge, but animals not carrying the virus will succumb. Following the foundation of a LCM-free stock some mice should be killed periodically and tested for the presence of virus. (If the animals are already being used for intracerebral inoculation work, this would itself provide a continuing control.)

E. MURINE ENCEPHALOMYELITIS

Three different, but related, viruses have been isolated from cases of encephalomyelitis in various stocks of laboratory mice: TO (Theiler's mouse poliomyelitis) which experimentally causes flaccid paralysis due to destruction of nerve cells in the ventral horns of grey matter of the spinal cord, and the FA and GD VII viruses. Infection is inapparent unless disease is provoked by various experimental procedures. The morbidity rate in colonies is extremely low (Theiler and Gard, 1940; Magnus and Magnus, 1949; Feltz *et al.*, 1953.)

Macroscopic and microscopic lesions are minimal and virtually confined to the central nervous system. Virus can be isolated from infected animals from the central nervous system, liver, spleen, and particularly the contents of the small intestine. Neutralizing antibodies are produced, and are best demonstrated by the inoculation of TO-free mice by the intraperitoneal route. Neutralization tests are difficult to demonstrate with stock from infected colonies unless the intracerebral route is used.

Diagnosis is most readily established by the inoculation of suspensions of small-intestine contents (treated with ether or streptomycin and penicillin) intracerebrally into 5-day-old mice. Signs of paralysis in the hind legs occur in positive cases 5 to 10 days after inoculation.

Control of this infection must depend upon the founding of a TO-free stock of mice derived from the offspring of old breeding females which have eliminated the virus, or from young fostered on to known TO-free foster parents, or on to rats. For much laboratory work the presence or absence of TO virus has no significance, but for serial passage studies it can become extremely important. The paper by Melnick and Riordan (1947) illustrates the pitfalls into which a stock carrying TO virus can lead.

F. DEMYELINATING DISEASES OF MICE AND RATS (JHM and DA virus of Pappenheimer *et al.*)

Cheever, Daniels, Bailey and Pappenheimer about 1949, in several papers, defined two viral conditions of mice each of which produced an unusual disseminated encephalomyelitis in which there was some specific attack on myelin. The original (JHM) viral infection was derived from two Swiss albino mice, 17-18 days old, which had developed flaccid paralysis of the hind

limbs. In experimentally infected animals, the incubation period was 4-8 days, shortening with repeated passage later to 24 h. In the earlier passages, paralytic signs prevailed, but as virus gathered virulence the signs were predominantly encephalitic, with incoordination, tremors and convulsions. The virus was recoverable from liver, spleen, lungs and kidneys as well as neural tissue, and was pathogenic for hamsters, sucking rats and other species.

The pathologic process was essentially massive destruction of white matter both in brain and spinal cord, with a tendency to spare the nerve cells. Another distinctive feature was the frequent occurrence of necrosis of the liver, not seen in Theiler's mouse poliomyelitis or other neurotropic infections.

Later, in the same colony, yet another paralytic condition of mice was found and a viral agent isolated—called at that time the DA virus. Unlike the JHM infection, there were no liver lesions. The relationship between these two viruses and Theiler's mouse encephalomyelitis virus was not settled, although they could be strains of the latter of low virulence.

Somewhat related to the above condition in mice, Pappenheimer also investigated a spontaneous demyelinating disease of adult rats, which although never proved to be due to a virus, yet deserves mention here merely because of the type of lesions found—a myelitis with specific predilection to attack the white matter of the spinal cord. No one else apparently has ever seen this disease, but like many matters concerning diseases of the nervous system of the laboratory animal, perhaps no one has ever looked. These notes are meant to stimulate those who are in a position to seek and find.

The original affected rats were 9 weeks old when they developed dragging of the limbs, and in a few weeks the paralysis was severe, but there were no tremors or other encephalitic signs.

Pathologically, there was a most extensive demyelination of the ventral and lateral funiculi of white matter at all levels of the spinal cord examined. All parts of the brain were free from lesions, and no significant changes were found in the viscera.

G. SALIVARY GLAND VIRUS INFECTIONS

Many studies have been made on salivary gland virus infection which has been found in mice, rats, wild rats, hamsters and guinea-pigs, and particularly the latter. It seems that host specificity has at least been proved in the latter and might well apply to other laboratory animals. It is an example of a latent infection, which produces no well defined pathological abnormality (of the salivary glands, and in particular the submaxillary gland). There is no evidence that it causes serious ill-health or any mortality, but in effect too little is known about pathogenicity. Occasionally, there is a mild inflammatory reaction in the gland, but more particularly there is constantly present intranuclear or (more rarely) cytoplasmic inclusion bodies in the ductal epithelium of affected glands. It is thus of importance to those who might be involved in examining

tissues from dead laboratory animals of all kinds—namely to be aware of the significance of such inclusion bodies. The virus seems to be widespread at least in guinea-pigs in the U.S.A. and Europe.

More recently Lyon *et al.* (1959) found cytomegalic inclusion bodies in both the intra- and extraorbital lacrimal glands (but not salivary glands) of adult male rats from eight separate colonies in the U.S.A. The section on an acute sialo-dacryoadenitis of rats described by Innes and Stanton (section XII) should be read, for this is a salivary gland disease without inclusion bodies but with gross pathologic changes. Further, since the date of describing the condition, which is unmistakable, it has been recognized in other colonies in the U.S.A. and in Europe (see Seifert, 1960).

H. CHRONIC MURINE PNEUMONIA

The name was given by Innes *et al.* (1956) to a disease in mice and rats as being preferable to the one in common use, namely enzootic bronchiectasis which manifestly only betokens an end-stage of a disease process which may take many months to develop. The condition in rats, in particular, is a disease of the lungs with an insidious onset, slow and protracted course and inevitably fatal outcome, if the rats are kept long enough. Mortality is thus low, but morbidity is extremely high in the ordinary colony, and is the outstanding disease hazard in any chronic experimental work with rats and which may vitiate or completely wreck experiments which have lasted a long time. In some recent experimental irradiation work and observations on longevity done by one of us, the disease occurred in around 100% of the 1-2 year old rats. Thirty years ago it is remembered also as being extremely common in rats on long-term nutritional experiments.

In earlier days, although recognized by most pathologists, a good deal of confusion was caused by the common isolation of PPLO organisms from infected lungs, and from that too loose assumptions were made on microbial causation. It was not until the work of Nelson (Rockefeller Institute) who showed that what was called bronchiectasis was very frequently a complex, mixed with another disease which he called infectious catarrh with its upper respiratory and middle-ear complications. He showed that they were different, first by eliminating the latter without eradication of the former. In summary, his later work proved that the chronic lung disease was initiated in the first place in baby rats by a virus which could be transmitted by the mother to her sucking young. Infection by direct contact from older rats to rats is possible if completely healthy rats are introduced into an infected colony. Thereafter the disease process is one of slow but increasing severity. During later development of the chronic lesions in the lungs, with destruction of tissue and later bronchiectatic cavitation containing caseous material, cell and mucus detritus, there is secondary invasion by a variety of micro-organisms. The following have been isolated at one time or other: *Bacillus muris*, *Br. bronchi-*

septica, *Pasteurella multocida muris*, *Streptobacillus moniliformis* (*Actinobacillus muris*) and *Mycoplasmatales*.

Nelson's papers should be read for an explanation of all work on aetiology, and are the only ones in the literature which give a balanced account of experimentation. The paper by Innes *et al.*, summarized below, is counterwise the fullest account of the pathology; it was based on examination of thousands of rats over the years.

All who have had experience with this disease express wonder that rats with such severe crippling destruction of lungs for a long time may not show very tangible clinical ill effects, although clearly there must be some. Comments on the limitations of clinical examinations of mice and rats were made at the beginning. In the late stages, the rats lose condition, have a staring coat, lose weight and there is unmistakable snuffling and wheezing. In the earlier stages, trials have proved that (as shown by autopsy) clinical observers were as frequently wrong as right in separating rats with normal lungs from those with diseased lungs.

All lobes of the lungs (the single left one and four on the right side) are equally susceptible. In some cases all right lobes are totally consolidated and the left lobe, which has more volume, is free. An affected lobe has a cobbled surface and is rubbery. The colour can be grey or red. Minute disseminated foci can also dominate the picture. Sooner or later, these lesions proceed to the formation of large nodular caseous or purulent masses which protrude from the surface of the lungs. On cutting into the lungs of late cases, bronchiectasis is then clearly apparent by the production of irregular cyst-like spaces, i.e., dilated bronchi and bronchioles filled with caseous material, pus, detritus and mucus. Pleural adhesions and empyema are rarely seen. Finally, the process may be so severe as to present, on section, a whole lobe or lobes composed of multilocular cavities surrounded by collapsed compact lung parenchyma.

Without giving too many details of microscopic appearances, the picture can be summarized somewhat in its development. Lungs from rats derived from colonies known to be free from this disease contain, and develop, little or no lymphoid tissue even into old age. In diseased colonies the appearance of massive cuffs and sleeves of lymphoid tissue in rat lungs, visible under a dissecting microscope, is of common occurrence, and long before there may be pneumonitis of any description. Consequently, there was a feeling by Innes *et al.* (1956) that here indeed was the incipient stage of a very chronic disease. Thereafter, in a rapid sequence of events, there is interstitial pneumonitis, alveolar collapse, perivascular inflammation, lymphoid hyperplasia, and bronchial fibrosis leading to increased rigidity of the bronchial tree. Plugging of the latter leads in the end to dilatation of the distal parts of bronchial passages.

As has been well demonstrated, the disease is completely resistant to all

known forms of chemotherapy—drugs or antibiotics—and it is a waste of time trying such methods out in any large colony. The only way, and an important one, to provide healthy rats free from this chronic insidious disorder (which almost precludes using such rats for long-term experiments over a year or so) is to eliminate it by starting with caesarean-derived stock and keep the colony in strict isolation thereafter. In the U.S.A., even this method has not prevented the reintroduction of the disease (virus) into such clean colonies, but the mode of transmission has never been proved or explained.

Many years ago Nelson showed that the same disease occurred in wild mice as well as laboratory mice and the descriptions in general given above might apply. As more mice are used for acute experiments, at an early age, than for chronic work, perhaps the chronic lung disease has not become so well recognized by the experimentalists who use mice. The fact that both mice and rats are susceptible and cross-transmission possible, simply indicates that both species should never be housed together, nor should colonies have "clean healthy rats or mice" housed in the same building as mice and rats bought from commercial sources. The latter, however, seems to be a common practice.

IV. Protozoan Infections

A. Eperythrozoönosis

Eperythrozoön coccoides was first described in 1928 in the blood of mice and is essentially an inapparent disease showing no external signs of infection. A small proportion of mice from an infected colony may show this organism in blood smears but normally infection is not rendered apparent unless a splenectomy has been performed. Accounts of the disease in mice are given by Derrick *et al.* (1954), Marmorston (1935), McCluskie and Niven (1934). McCluskie and Niven found increased numbers of *Eperythrozoa* in mice within 48 h of splenectomy, Marmorston within 6 to 7 days. *Eperythrozoa* appear as small disc- or ring-shaped, dark-staining, structures adhering to the surface of red cells in Giemsa-stained blood smears. They show a tendency to attack polychromatic cells, and organisms can be detected up to 4 months after splenectomy or inoculation. There is a moderate anaemia but the disease is usually self-limiting, ending between 12 and 21 weeks after inoculation. *Eperythrozoa* produce no disease if inoculated into uninfected mice, but subsequent splenectomy results in the appearance of organisms in the blood. Infection occurs regularly if splenectomized mice are inoculated. Irradiation appears to increase the incidence of these parasites in the blood of normal mice. Not all strains of mice are affected; Marmorston found three out of eight colonies examined to be infected. For full descriptions of the parasites and their life cycle, refer to texts on parasitology and protozoology.

At autopsy the only macroscopic abnormality consists of enlargement of the spleen, which can increase until it is about 2% of the total body weight. Enlargement appears to be most marked between 7 and 28 days post-inoculation (Derrick *et al.*, 1954).

Prior to the work of Gledhill and his colleagues on mouse hepatitis virus (MHV) it was considered that this organism merely functioned as an annoying contaminant in certain types of experimental work. Their studies with MHV have shown that the presence of *Eperythrozoön coccoides* in mouse stocks can markedly affect the severity and outcome of infection with other agents. A similar enhancing effect of concurrent *Eperythrozoön coccoides* infection upon lymphocytic choriomeningitis has been recently demonstrated (see Seamer *et al.*, 1961.)

Diagnosis depends upon the examination of serial blood smears prepared from splenectomized mice and recognition of the parasites inside red cells. Control depends upon the use of stock demonstrated to be free from infection. Possibly chemotherapy will effect a useful control. Weinman (1944) suggests that the treatment of pregnant females should give an *E. coccoides*-free strain. He suggests the use of neosalvarsan, 0·0025 g for a 15- to 20-g mouse, or sulpharsphenamine 0·0025 g followed a day later with 0·00125 g.

B. BARTONELLOSIS

Both *Eperythrozoön* and *Bartonella* (*Hemobartonella*) *muris* infections occur in mice and rats but the former predominates in mice and the latter in rats. Nevertheless, there are some reports of *Bartonella* infection in mice. McCluskie and Niven reported the finding of *Bartonellae* in a few splenectomized mice; splenectomized mice inoculated with *Bartonellae* developed a severe and fatal anaemia. Marmorston noticed a severe bartonellosis in mice after an *Eperythrozoön* infection had subsided—in this case infection lasted only a few days, with no anaemia and non-fatal outcome. Again, reference should be made to Weinman (1944).

C. COCCIDIOSIS

Coccidial infections of laboratory mice and rats are neither widespread nor common in contemporary mouse colonies. The few cases seen usually occur either in old mice or under conditions of poor hygiene. Reference should be made to standard textbooks for details of the life cycles of these parasites. A brief but valuable review of the organisms considered in this section is given by Heston (1941).

Infections in mice with *Eimeria falciformis*, *Cryptosporidium muris* and *Cryptosporidium parvum* have been reported but are rare. *Eimeria falciformis* produces oöcysts 16-21μ × 11-17μ which can be found in the epithelial cells of the large and small intestines. The *Cryptosporidia* referred to do not appear to be intracellular parasites but are found attached in the case of

C. *muris* to the gastric glands in the stomach and in *C. parvum* to the cells lining the intestinal villi. Actual disease due to these parasites is very rare. (A useful account of these and all other internal parasites of mice is to be found in Habermann *et al.*, 1954.)

D. *Klossiella muris* INFECTION

Infection with this coccidium may be detected only in old mice, such as are often kept in cancer colonies. Signs of infection are confined almost entirely to the kidney, which show a very pale mottling of the surface by minute greyish specks. Spore cysts, containing about twenty-five sporocytes, can be found in the tubular epithelium. These spore cysts (about 13-16 μ in diameter) are released into the tubules and enter the urine. The disease is relatively benign, cases of obvious sickness and death due to this organism being rare. No precise control measures are known, but obviously with a pathogen excreted in the urine of infected animals general hygienic measures should exert a useful control over this disease.

E. SARCOSPORIDIOSIS

This condition in man and animals was reviewed in its entirety by Eisenstein and Innes (1956), and there is no significant publication since then which would cause any alteration of the statements made at that time.

Sarcosporidiosis is an infection, mainly of the skeletal muscles but also cardiac, lingual, laryngeal, pharyngeal, palatal and oesophageal muscles, with protozoan organisms of the genus *Sarcocystis*. These organisms occur in all domesticated animals, man, wild and laboratory animals; the suggestion that they are fungi has never been substantiated. There is usually a benign association between parasite and host. The parasite was first described in 1843 by Miescher, hence the designation of the psorosperm sacs as Miescher's tubes, and we know as little about the mode of transmission of the infection as he did. In mice and rats the infection can only be identified, usually accidentally, by histological examination of muscle from any region, when the parasites stand out as (hematoxylin-eosin) blue-stained encapsulated granular bodies inside individual muscle fibres, and mostly with no reaction round. The psorosperm sacs contain sporoblasts which turn into spores and with maturation the sac ruptures with liberation of the spores to infect other muscle fibres. The centre may degenerate or calcify, and some workers claim at this stage that there may ensue an inflammatory reaction which results in the so-called chronic sarcosporidial myositis of German workers. On more rare occasions the parasites may produce a very acute reaction, and such a lesion in a monkey is shown by Hadlow (in Innes and Saunders, 1962). The Miescher tubes on occasion have been mistaken for other parasites.

Although this infection has such an ubiquitous spread amongst mammalian hosts, it is certain that over the past 30-40 years the general picture has

changed. One of us can remember, in examination of mice and rats some 30 years ago, that it was rare (in England) ever to see skeletal or cardiac muscle in which one or many sarcosporidia were not found. In the last decade many mice and rat colonies in the U.S.A. have been noticed to be entirely free and this was proved in a deliberate histological survey mentioned in Eisenstein and Innes. As it rarely causes serious ill-health in mice and rat colonies, and almost never any mortality, it is of little practical importance; it still requires mention in such a text as this, merely to indicate that the parasites and the infection should be recognized for what they are in routine *post-mortem* examinations of mice and rats.

F. TOXOPLASMOSIS AND ENCEPHALITOZOÖNOSIS

Both these diseases can be conveniently discussed together. For detailed descriptions of the parasites see standard texts on parasitology. The topic of the infections as they affect the nervous system and muscle are fully reviewed by Innes and Saunders (Chapters V and IX), which obviates the need here to cite any of the very extensive bibliographies on either disease. All references to the authors cited below will be found there, or in a valuable complete bibliography kept up to date by Eyles and Frenkel on toxoplasmosis. The literature on encephalitozoönosis is given in Innes *et al.* (1962).

Toxoplasma gondii was first found in birds and then mammals over 50 years ago, but it was not until 1937 that the parasite achieved prominence as a cause of human encephalomyelitis in new-born babes. It has now been found in most of the domesticated and laboratory species, either as an occult infection or as an actual disease, and in some species it is of economic importance. Two forms of the parasite are recognized. There is the proliferative form seen in acute infections. It is a crescentic organism about 2-4 μ in width and 4-7 μ in length possessing a central nucleus and a few minute glycogen granules. It multiplies by binary fission and is liberated when a host cell ruptures, and other cells are then invaded. Residual or chronic infection is maintained by formation of the pseudocyst or cyst which is about 30-60 μ and which has a tough resilient cyst wall, stainable by the periodic acid Schiff method. When intact, such cysts may not cause any tissue reaction and frequently localize in skeletal and cardiac muscle as well as elsewhere.

While a great deal of the information on toxoplasmosis has come from experimental work using laboratory animals, reports on outbreaks of spontaneous disease or infection in mice and rats are rare. It is important to keep the disease in mind, for experimentally at least it has been proved by Beverley (1959) that congenital (transplacental) infection in mice is possible over several generations.

Encephalitozoön cuniculli was the name given by Levaditi to a parasite he found in rabbits in 1924, though the parasite had been found in the brain of

mice and rabbits long before then but had sometimes been called by another name. Since that date reports have multiplied and we know that the encephalitozoön occur in a large variety of species either as a latent infection or as a disease. In rabbits it can be a serious disease producing clinical effects such as circling and paralysis and associated with inflammation and granulomata in the brain. The organism is much smaller than toxoplasma, being about 2·5 $\mu \times 0{\cdot}5$-1 μ and is ovoid with a small chromatic granule. Both stain with Giemsa, but encephalitozoön are picked out with clarity by Goodpasture's carbol fuchsin method. Although forming in groups like the cysts of toxoplasma, they do not have a proper cyst wall which is stainable. Most reports of encephalitozoönosis in mice or rats have been isolated sporadic infections. However, Innes *et al.* (1962) described an outbreak in an isolated colony of mice started with caesarean-derived stock which necessitated the sacrifice of the entire colony. This infection must have been in the first place transmitted by transplacental routes and passaged through several generations. No clinical signs were seen and the mortality was almost nil. The lesions were essentially those of a low-grade meningoencephalitis affecting all parts of the brain in which organisms were found with ease. This infection was experimentally reproduced by intraperitoneal injection of tissue emulsions only after cortisone treatment of mice. The condition is one of importance in breeding colonies for it can be an occult infection and clearly be potentially transmitted as a dual infection if mice with encephalitozoönosis are being used for virological studies. Evidence is available that this has indeed happened. An apparently identical occult infection with encephalitozoön seems also to have been proved in the laboratory rat in England by Atwood (private communication).

V. Mycotic Infections

A. Ringworm

Ringworm infections in mouse and rat colonies seem to be less common than they were at one time, but sub-clinical infection is probably more widespread than is usually suspected. Infections with *Trichophyton* and *Microsporum* spp. are most common.

Often infected colonies show no clinical signs of the disease, but staff handling the animals contract ringworm from time to time. Clinical disease is usually evidenced by the presence of bald patches on the backs and necks of affected animals. The skin in these areas is thickened and incrustated, and the hairs at the edges of the lesions are brittle and easily removed. Badly affected animals show a lack-lustre coat. In some outbreaks there is formation of a hard, crust-like scutulum on the backs of affected animals, as described by Blank (1957) in an outbreak due to *Microsporum quinkeanum*. At autopsy the only lesions found are those found on the skin and pelage.

Experimental transmission of disease can be achieved readily—either

directly, from animal to animal, or by lightly scarifying the skin of a mouse with a culture of the fungus.

Diagnosis of fungal infections can usually be established by microscopical examination. Hairs are removed from the edge of skin lesions, mounted in 10% potassium hydroxide and examined under the microscope. Chains of spores, measuring 3-6 μ in diameter, can be seen in the medulla of infected hairs. Species identification of the fungus requires cultural studies, which are readily made by planting affected hairs on the surface of Sabouraud's medium. After 3 or 4 days tufts of growth appear which enlarge and take on a chalky appearance as spores are formed. Microscopic examinations of such cultures will identify the species (see Figs. given by Blank, 1957).

This disease is particularly important because of the risks of transmission to animal staff. Ringworm in animal technicians is too common for it to be neglected. Valuable accounts of this disease are given by Parish and Craddock (1931), Blank (1957) and Dolan *et al.* (1958).

Control is difficult. Individual cases should be culled and so should cage contacts of such animals. The disease is often self-limiting and infection of individual animals clears up with no specific treatment being given. Widespread infection, particularly where it is associated with subsequent human cases, is probably best controlled by a simple slaughter policy and a refounding of the colony from fresh stocks, following complete and thorough disinfection of the animal rooms and equipment. Individual cases and cases among small numbers of animals will probably be cured by treatment with griseofulvin which is conveniently incorporated in the diet so that the animals get a dose of approximately 60 mg per kg live weight. Gentles (1958) has described the development and use of this drug for the treatment of ringworm infections.

A recent case of spontaneous infection in a mouse with *Cryptococcus neoformans* has been described by Sacquet *et al.* (1959). They observed multiple splenic and renal abscesses and cryptococci were found in the glomeruli and renal tubules.

VI. Helminthic Infections

Helminthic infestations of laboratory mice and rats may not be important in the sense of causing mortality, but there is no doubt that, as in all other mammalian species, alimentary and other helminthic infections can be a cause of deterioration in general health and condition. It is important that they be recognized, for methods of treatment are available. In colonies derived by the caesarean method and fostered on to clean or germ-free parents, alimentary helminths cannot, clearly, be transmitted to the sucking rats or mice. Hence, as has been found, starting with such breeding stock free from worms there is no difficulty in keeping the colony free from helminths and from protozoan infections as well.

For a description of the helminths mentioned below, see standard texts of parasitology, such as Lapage, as well as the specific articles mentioned.

A. PINWORM INFESTATION (OXYURIASIS)

Infestation with the pinworms (oxyurids) *Syphacia obvelata* and *Aspicularis tetraptera* (and to a much lesser extent *Heterakis spumosa*) is probably more widespread than is usually thought in mouse and rat colonies. Careful survey of stocks will often reveal a far higher incidence of infestation than one might expect.

There are no clinical signs referable directly to infestation with *Syphacia* and *Aspicularis* spp., but gross nematode infestation is often associated with various forms of malfunction of the gut. Occasionally, solid plugs of pinworms can be found in the gut adjacent to hard impacted faecal pellets, intussusception loops, and in cases of rectal prolapse. It is difficult to say which is the primary and which is the secondary cause of the conditions observed.

At autopsy there may be signs of gut inflammation, but it is frequently possible to find large numbers of pinworms in the caecum and colon with no associated macroscopic lesions.

The life cycle of *Syphacia obvelata* is well described by Chan (1952). Habermann *et al.* (1954) give useful, if brief, descriptions of all three parasites. See also Heston (1941).

The life cycle of these nematodes is completed within the gut of a single animal, with infective eggs being found in the faeces between 15 and 30 days following ingestion of eggs. Cross-infection occurs by way of faecal contamination of food and drinking water and also (and this increases the difficulty of controlling the natural infection) by direct mouse to mouse transfer. Mature worms deposit eggs on the perianal skin of infected animals from where they are readily ingested by licking by another animal (see the valuable account of Sasa *et al.*, 1962).

Diagnosis of pinworm infestation is made either by egg flotation methods, Scotch-tape anal smears, or after autopsy. The latter, which is undoubtedly the most reliable, is made by removing the caecum and large intestine and cutting them open in a petri dish of water and gently removing and suspending the intestinal contents. Over a dark background small white pinworms between 1 and 5 mm long are readily seen with the naked eye, or under a low-powered microscope.

Salt flotation methods can be used for the examination of faeces for the presence of eggs. Three or four faecal pellets are collected from individual animals and emulsified in saturated salt solution in a 3 in $\times \frac{1}{2}$ in test-tube. When an even suspension has been made the test-tube is stood upright, filled to the brim with more salt solution and allowed to stand for 30 to 60 min. After standing, a clean cover-slip is inverted over the top of the tube to pick up a small drop of salt suspension, which is then examined under a low-

power objective. Eggs can be seen at ×20 magnification and the two major species identified (see Habermann *et al.*, 1954).

Eggs deposited on the perianal skin are removed by sticking a small square of Scotch tape over the skin in this area, removing it, and examining it on a slide under a microscope.

Control and maintenance of infection at a low level is achieved by routine hygienic measures, but chemotherapy is recommended when infestation becomes unusually extensive or obvious. A variety of agents has been used for this purpose, but phenothiazine and piperazine compounds are the most widely used. Phenothiazine is mixed with the food at the rate of 0·5 g per 20 g mouse food. Piperazine citrate and piperazine hydrate can also be given in the food, but are more simply and conveniently administered in the drinking water at a rate of 200 mg per g body weight (approximately 3 g piperazine hydrate per litre drinking water). Treated drinking water is given to the animals for 7 days, followed by 7 days with untreated water, followed by a further 7 days with treated water. By this method the worm burden of a colony can be very considerably reduced. As emphasized by Hoag (1961), it is important when carrying out this kind of eradication programme to treat one entire room on a single occasion, otherwise residual infestation and contamination will result in the recurrence of widespread infection (see also Habermann and Williams, 1958).

B. TAPEWORM INFESTATIONS

Tapeworm infestations are less common than pinworm infestations in mice, but occasionally they can be troublesome. Two species predominate—*Hymenolepis nana* (syn. *H. fraterna*) and *Hymenolepis diminuta*. Brief accounts are given by Habermann *et al.* (1954).

Clinical signs are rare, although gross infestations may lead to emaciation and general loss of condition. At autopsy, grossly infested animals may show some enteritis, but macroscopic lesions are minimal. A diagnosis can be established by the examination of faeces for eggs or by dissecting out the gut and identifying mature tapeworms.

It should be noted that man is susceptible to infestation with *H. nana*, although it is still not clear whether the strains of murine origin are markedly pathogenic for man. Nevertheless, care should be taken when handling infected animals.

It is possible that *H. nana* has a direct life cycle, i.e., no intermediate host is required. This makes it more difficult to control infestation within a colony. *Hymenolepis diminuta* does not appear to have a direct life cycle and requires an intermediate host, such as cockroaches, beetles, fleas or mealworms. In this case colony infestation is obviously controlled by attacking these intermediate hosts. The use of chemotherapy has been described by Habermann *et al.* (1954).

Taenia crassicolis infestation is occasionally found in mice and rats. The cystercercoid form of this tapeworm (whose normal host is the cat) develops as a cyst in the liver from which it is readily removed at autopsy. Hepatic sarcomata are said to arise in infested mice (see Heston, 1941). Infestation with this tapeworm is not usually extensive, but its presence is indicative of contamination of mouse foodstuffs with cat faeces, which may occur in the animal house or the miller's stores see the account by Duffil and Lyon, 1960).

VII. External Parasitic Infestations

Mice and rats may carry a number of arthropod parasites in their pelage, but only three are common.

A. LICE AND FLEAS

The louse, *Polyplax serrata*, is the usual species found. Infestation of a very low-grade nature might well be missed, but if a colony is infested at all it usually becomes obvious on mice and rats which become sick. Lice appear to multiply considerably when the animal's general state of health is poor—as might follow experimental procedures, for example. A very heavy louse infestation may itself result in the general loss of condition of the animal. The parasites are readily detected by the procedures described below for mites. Some control can usually be obtained by dusting the whole cage and its inhabitants with one of the many proprietary insecticides available containing γBHC (benzene hexachloride), e.g., gammexane, lindane, pyrethrum or, provided it is used sparingly, DDT. Complete eradication is unlikely to be achieved by this method but it is quite possible to reduce infestation to manageable proportions.

Polyplax serrata is a blood-sucking louse and is potentially capable of spreading blood-borne disease from mouse to mouse. It has been implicated in the spread of *Eperythrozoön coccoides* (Heston, 1941).

With colonies practising a regular and efficient hygienic routine, flea infestations (which can be due to a variety of species, see Heston, 1941) should be virtually unknown.

B. BEDBUGS

Bedbugs (*Cimex lectularius*) occasionally become a nuisance in mouse colonies and a very heavy infestation can reduce the general standards of health of the mice. This pest spends the daytime hidden away in crevices and cracks, and emerges at night to feed on any animal species available, and eradication in old or badly designed animal rooms is a difficult task. Control depends on the thorough cleaning and disinfestation of each room affected and dusting all likely hiding places with a suitable insecticide.

C. MITES

Two species of mites occur commonly in mouse colonies: *Myobia musculi* and *Mycoptes musculinus*. Flynn (1955) describes the frequent occurrence of

these two species and also *Radfordia affinis* and *Psorergates simplex* on mice in America. The latter species is probably rare in the U.K., having been described only once recently by Cook (1956), but *Mycoptes musculinus* and *Myobia musculi* are very common in this country (see Gambles (1952) in which are to be found some excellent drawings of these two species.)

Probably most colonies of laboratory mice carry the two myobid mites. Usually there are no signs of clinical disease and frequently animal staff are unaware of the existence of infestation, although it is a simple procedure to examine mice for the presence of these mites. Heavy infestations may lead to the formation of scabby, sometimes numerous, skin lesions and a marked loss of hair. Presumably such lesions are the result of the animal scratching at areas irritated by the pests. *Psorergates simplex* gives rise to small whitish nodules (usually on the ears) which, when sectioned or dissected, prove to be small infoldings of the epidermal tissue in which the mites have nested.

Diagnosis is readily made by microscopic examination of hairs removed from the neck and belly region and mounted in 10% potassium hydroxide. Mites and eggs (and also lice) in various stages of development are easily seen under a low-power lens. Flynn (1955) describes a procedure for examining animals at autopsy. The mice are laid out immediately after killing on sheets of black paper and as the body cools the mites vacate their host and can be seen against the dark background. This method is satisfactory only if the infestation is very heavy. Lower levels of infestation are more readily diagnosed by simple microscopical examination of a few hairs.

Partial control of mites can be gained by dusting boxes and their inhabitants with gammexane preparations as described for lice, but the degree of control is not always very great. Better control can be obtained by dipping infected mice into warm suspensions of suitable insecticides. A variety of these have been described. Tetraethylthiuram monosulphide (tetmosol, I.C.I., England) diluted to 1·4% is claimed by Cook (1953) to be effective. Stoner and Hale (1952) describe the use of a 0·2% suspension of DMC in 50% ethyl alcohol. The alcoholic solution appears to increase the effectiveness of this miticide, but when dipping animals in this suspension it is most important that they are kept very warm whilst they are drying off after the dip (DMC = di(*p*-chlorphenyl) methyl carbinol). Dibutyl pthalate, painted neat over affected ears once or twice, was found by Cook (1956) to be quite effective in controlling *Psorergates simplex*. A recent account (Bateman, 1961) reports the use of a dip containing DMC and tetmosol. This dip, being aqueous, is easier to handle than that employing DMC and alcohol. Bateman's dip consists of 2 g of DMC dissolved in 3 g of ethanol, mixed with 67 g of Tetmosol in a litre of warm tap water. (DMC is obtained from Sherwin Williams Coy., Cleveland, Ohio.)

When dipping mice in any of these suspensions it is important that the dip be maintained at roughly blood temperature and that the animals be allowed to dry off thoroughly in a warm atmosphere. Strains seem to vary in the ease

with which they accept such treatment, but with care all strains tried by the writer, and even mice between 2 and 3 weeks of age, survive the treatment. For complete eradication the treatment should be given twice, over an interval of about a fortnight. To ensure thorough wetting of the animals' coats and penetration of the acaricide to the base of the hair it is advisable to give the animal a preliminary dip which conditions the coat to receive a second dip given 30-60 min later. Bateman's technique appears to cope with *Mycoptes musculinus*, *Myobia musculi* and *Polyplax serrata*. Finally, it is important that if a dipping programme is undertaken it should be thorough. The aim should be to clean at least one entire room at a time, and dipped animals should be replaced in fresh, clean, sterile cages after dipping.

VIII. Deficiency Diseases

Although the rat has been the traditional animal of choice for experimental nutritional studies, it is doubtful if authenticated cases of spontaneous disease caused by any deficiency of the many vitamins has ever occurred apart from vitamin E. The same probably applies to mice. Even if mice and rats are not given an optimal diet (see Chapter 2), they are usually given a diet which, if by chance it is deficient in any factor, is never so to the point that actual clinical disease is produced. As a general source of information on the pathology of experimental deficiencies produced in rats and mice, reference can be made to the comprehensive book by Follis (1958).

A. Vitamin E Deficiency

The vitamin E content of a diet is likely to decrease (sometimes very much) as the result of breakdown caused by the presence of rancid fats in the diet. Cod-liver oil is particularly liable to cause this breakdown. Bruce (1950) describes such an event in a diet containing 2% cod-liver oil. She found that reduction to 1% prevented the deficiency, but nowadays this particular diet was been modified slightly and no longer contains any cod-liver oil. Evidence of a vitamin E deficiency usually shows first as a fall in productivity, and it can be disastrous. This fall in productivity is due to (i) a fall in the conception rate and (ii) a fall in the weaning rate of those mice born. In the outbreak described by Bruce the weaning rate dropped to less than 50%. The pre-weaning losses were due to lactational failure, neglect or cannibalism. Other losses were caused by abortions, and others (which would not normally be recognized) were due to resorptions. In newly weaned mice paralysis may occur, but this is apparently not as frequent in this species as in the rat. Muscle lesions can be found in mice over 16 days of age at autopsy. Male mice on an E-deficient diet show a marked loss of fertility and can become completely sterile; there is a noticeable degeneration of the seminal epithelium. In male mice, but not in rats, this degeneration is reversible and replacement

of the E-deficient diet with one adequately supplemented results in regained fertility.

Control of this disease is obvious once it has been diagnosed. It may be useful to add to normal diets a pre-mixed vitamin supplement including vitamin E, but it is equally important to avoid the use of cod-liver oil and other substances which, going rancid, could oxidize the vitamin present.

The effects of vitamin E (tocopherol) deficiency on production of dystrophic changes in the skeletal muscle of all laboratory animals are fully discussed by Hadlow (in Innes and Saunders, 1962). He says that the severity of the disease and the ease with which it can be produced varies with the species and the age of the animal involved; it occurs, in order of frequency, in rabbit, guinea-pig, rat, mouse and monkey. The changes are essentially those of a non-specific myodegeneration—waxy or hyaline changes, lumpy cleavage, loss of striation, proliferation of muscle nuclei and an accompanying inflammatory reaction.

Hadlow also discusses work of Einarson on the very chronic effects of tocopherol deficiency on the nervous system of experimental rats. These observations cannot be of interest in this compilation, for the length of time taken for adult rats to survive a severe deprivation of vitamin E deficiency and to produce the lesions which Einarson describes makes it virtually impossible for such a disease to occur spontaneously in a rat colony kept under modern conditions.

IX. Intoxications

A. CHLOROFORM POISONING

Low concentrations of chloroform vapour in the air can be extremely toxic for adult mice of certain strains. This fact is not always realized and has led (in the experience of A.A.T.) to a number of small "epidemic" outbreaks with high mortality which was at first unexplained. Because of this risk, chloroform should never be used in the animal house for killing animals. The use of a single small killing jar with chloroform in a room full of mice is sufficient to cause considerable losses among susceptible males. At least three incidences of this type have been described in the literature (Deringer *et al.* (1953); Hewitt (1956); and Shubik and Ritchie (1953)).

Chloroform intoxication is usually first suspected when there is a sudden outbreak of losses among male mice. Adult female mice, young male mice up to 30 days of age and castrated male mice are insusceptible. Gonadectomized mice can be rendered susceptible by treatment with a variety of hormones (Culliford and Hewitt, 1956).

At autopsy abnormalities are seen only in the kidneys, which take on a speckled white appearance varying in extent from animal to animal. Histologically, the kidneys show extensive necrosis of the proximal and distal convoluted tubules, but no damage to glomeruli, capsules, the straight part

of the loop of Henle, collecting tubules, renal papilla or the renal pelvis. Necrosis is extensive, resulting in complete loss of tubular epithelium. Prior to death there are few or no specific signs of illness although urine analysis would show excessive protein. A slight degree of necrosis in the liver may be found in male mice dying from acute chloroform exposure, but this is not found in mice surviving for 24 h or more. Susceptible male mice have been found in the following strains: DBA,, WH (a heterozygous albino), C3H, A and HR. Deringer, Dunn and Heston found male mice of strains C57BL, C57BR/cd, C57L and ST to be resistant to accidental exposure. While, in the accidents recorded in the literature, inbred strains of mice appear to be the only ones susceptible to chloroform poisoning, experimental work at Carshalton and elsewhere demonstrates that kidney damage occurs in mice of non-inbred strains. The lesions in such mice may not result in death, but the inclusion of accidentally exposed animals in an experiment could seriously confuse any results obtained. Small foci of calcification at the medullary border have occasionally been found in female mice previously exposed to chloroform.

The finding of sudden high mortality rates exclusively among male mice should suggest at once the possibilities of chloroform poisoning (related compounds such as carbon tetrachloride similarly give rise to renal necrosis). At autopsy the finding of lesions only in the kidney is suggestive and histological examination of the renal cortex should confirm the diagnosis. Control measures are obvious. The use of chloroform for killing animals or for any other purpose in or near rooms housing mice should be strictly forbidden.

B. DDT

DDT (2,2,-bis(p-chlorophenyl)-1,1,1-trichloroethane) is now used widely in animal houses, but there is a toxic risk associated with the use of this substance. Cases will be rare, however, unless there has been gross misuse of insecticides containing DDT. Valuable accounts of studies on the toxicity of DDT to mice and other laboratory animals are given by Neal *et al.* (1944), Woodard *et al.* (1944), Neal *et al.* (1945), Cameron and Burgess (1945).

Experimentally, toxic signs can be demonstrated by repeated cutaneous application of about 2 mg of DDT over periods of 1 to 3 weeks, a single dose *per os* of about 500 mg per kg, feeding of food containing about 500 ppm DDT over a period of about 3 weeks, or exposure to aerosols 3 times a day over a period of 4 weeks with a concentration of 0·183 mg of DDT per l of air. Exposure to aerosols of higher concentration produced death in varying proportions of the exposed animals.

In DDT-poisoned animals clinical signs include hyper-irritability and a decrease in food consumption. At autopsy animals exposed to marginally toxic doses of DDT show few or no pathological changes. Animals exposed

to higher concentrations may show enlarged, fatty livers with varying amounts of congestion, fatty changes in the proximal and distal convoluted tubules and occasional hyaline casts in the kidney, haemosiderosis in the spleen, and chromatolysis, occasional vacuolation, and karyolysis of neurons of the spinal cord (Cameron and Burgess, 1945). The signs and lesions observed at autopsy vary considerably between individuals. Mice are more susceptible than rats and guinea-pigs, and dogs and monkeys are considerably more resistant.

The lesions and signs of toxicity described by the authors quoted were observed only in animals which had received what were in fact very large doses of DDT. Neal *et al.* (1944) point out that for entomological purposes one requires a DDT aerosol concentration of about 0·004 to 0·007 mg per l of air. Their own inhalation experiments which produced toxic signs used a concentration of between 19 and 33 mg per l, i.e., some 3,000/4,500 times the required concentration. From this it is obvious that when used as an aerosol the risks associated with DDT are very small. Similarly, when DDT preparations are used for direct application to animal coats for the control of mites and lice, the concentrations used are likely to be very much below the level at which toxic signs in the mice themselves would be observed.

C. γ-BENZENE HEXACHLORIDE

The various isomers of benzene hexachloride (α, β, γ, δ) have different insecticidal properties. In commercial preparations of benzene hexachloride it is the γ-isomer which is of interest. There appears to be far less information on the toxicity of this substance to mice than there is for DDT (see Furman, 1947). Experimental cases of benzene hexachloride poisoning show few abnormalities at autopsy beyond an occasional haemorrhagic gastritis and duodenitis. Before death the animal trembles sneezes and paws its nose; breathing may become laboured. Later the signs worsen and the animal has convulsive spasms.

Single doses of 80 mg γ-benzene hexachloride per kg body weight produced no effect, but 200 mg per kg killed two out of six mice. In a chronic experiment, involving the ingestion of a total of 45 mg of the γ-isomer over 30 days, eight out of ten mice survived with no apparent ill effects. In experiments involving the dipping of mice in a 5% suspension of benzene hexachloride, some mice died, almost certainly by ingesting γ-benzene hexachloride as they licked their coats following dipping. Mice prevented from licking their coats tolerated a 10% dip.

It follows from the observations described above that γ-benzene hexachloride, as used in the animal house under normal conditions, is not likely to constitute a very serious toxic hazard, though some care needs to be taken when it is proposed to dip animals.

D. HORMONE CONTAMINATION OF FOODSTUFFS

From time to time there have been reports of hormone contamination of mouse feeding-stuffs (see Paterson and Brimblecombe, 1959; Hadlow and Grimes, 1955; Hadlow *et al.*, 1955; Dorfman and Dorfman, 1955). Contamination appears to have been more frequent in the United States than in Europe, but obviously there is a risk wherever manufactured cube diets are used.

The account by Dorfman and Dorfman (1955) describes the detecting of oestrogen in food materials as a result of changes in uterine-weight/body-weight ratios during the course of oestrogen determinations. Oestrogen was found in corn and four commercial feedstuffs. Paterson and Brimblecombe describe contamination with hexoestrol, and Hadlow and Grimes contamination with stilboestrol. The onset of hormone contamination may be sudden; the outbreak described by Paterson and Brimblecombe was traced to a single batch of foodstuffs. Clinically, such contamination may be suspected when one or other of the following signs are observed. Hadlow and Grimes reported the occurrence of scrotal hernias in 90% of their breeding adult males. At autopsy the caecum and small intestine were commonly found in the scrotal sacks. Histologically, the testes showed varying degrees of atrophy. Among female mice a variety of changes may be observed, such as a decrease in the pregnancy rate, decrease in the average litter size, increased number of mice dead at birth or shortly after, poor lactation or failure of lactation, increase in the abortion rate, and young females in constant oestrus. The effect of these changes can be disastrous to a breeding programme and result in a colony's productivity falling to 50% or less of the normal value.

The clinical signs described, in the absence of any other more obvious aetiological agent, should suggest hormone contamination of foodstuffs. The diagnosis can be established by attempting to extract hormones from samples of foodstuffs. It is important to note that various strains of mice differ in their sensitivity to oestrogens so that, at least theoretically, it is possible to get all grades of effect on colony output from the complete failure of a breeding programme to a slight fall-off in productivity, which might not be readily detected or explained.

In attempting to control such outbreaks of disease, four possible sources of hormone contamination must be kept in mind. (i) A batch of foodstuff may be accidentally contaminated with hormone intended for a different feed mix, e.g., cattle. This appears to have been the case in the outbreak described by Paterson and Brimblecombe. (ii) Contamination by mixing mouse foodstuffs in a machine previously used for a batch of animal feedstuffs in which a hormone was deliberately incorporated. (iii) The use of meat scraps from poultry which have been reared with implants of hormone-containing pellets. (iv) Some plant material itself may naturally possess oestrogenic activity. If these four possible sources of contamination are kept in mind, then control measures are obvious.

X. Neoplastic Diseases

A. GENERAL

The topic of spontaneous tumours in the mouse and rat is of extreme importance, largely because these are the species mostly used for experimental cancer research all over the world. The literature is prodigious on both aspects and cannot possibly be covered here. However, because of its importance we cannot exclude such a field. As the intended readers of this book are not expert pathologists or cancer research workers, but those in charge of animal colonies, we have simply given some directional literature and discussed a few of the virus-produced tumours.

In most mouse- and rat-breeding colonies, the survival of males and females probably averages no more than about 5 months, or five litters; this age is such that only a low incidence of spontaneous tumours may be found. This should not be neglected and animal attendants ought to be trained to recognize that all lumps anywhere on the body may be cancer and that they should be examined histologically. Only in this way will a true picture of the cancer incidence in any given strain be recognized.

The best all-round description of tumours in all laboratory animal species is in Cohrs *et al.* (1958) by Dobberstein and Tamaschke, and from their chapter all available references on spontaneous tumours can be found (for other important references see Lippincott *et al.* (1942), Wells *et al.* (1941), Slye *et al.* (1921) and Dunn (1954)). As we have stressed in the section on inherited diseases, and so also with cancer, there is a tremendous difference in susceptibilities to different types of neoplastic diseases as well as different organ susceptibility. This will be found by perusal of the Jackson Memorial Laboratory publications on genetically standardized mice.

Whereas tumours of the skin, genitalia, eyes, etc., may be found by visual observation of living mice and rats, obviously cancer of the viscera can only be detected *post mortem*.

B. VIRUS-INDUCED LEUKAEMIAS

Gross (1951) reported the production of leukaemia in C3H/Bi mice derived from Bittner's sub-line by the inoculation of cell-free extracts of leukaemic tissue. Miller (1960) questions the evidence for virus formation of this leukaemia. Another virus described by Friend (1957, 1959) produces a proliferative disease that may lead to leukaemia. This was discovered in young Swiss mice. Specific neutralizing anti-sera could be prepared which prevented infection in mice, and a formolized vaccine prepared from filtrates could also give protection against disease in inoculations.

It is difficult to assess the role of these virus infections in mice used for many laboratory purposes, but obviously workers interested in a wide variety of cancer studies must be aware of such leukaemia-inducing viruses and be

prepared to consider their relevance to their own particular studies. Reviews of this topic are to be found in Anon. (1960) and Gross (1960).

C. MAMMARY TUMOUR AGENT (BITTNER AGENT)

Infection with mammary tumour agent (MTA) may lead to a greatly increased incidence of mammary tumours in female mice. This agent was first discovered by Bittner in 1936; reviews are given by Andervont (1946) and De Ome (1962). The discovery of a virus aetiology of an undoubted cancer stimulated a considerable amount of work in this field and mouse mammary tumours have probably been more widely studied than any other form of animal tumours.

This disease affords a good example of vertical transmission of the aetiological agent, since spread from one generation to another is effected almost entirely by means of the mother's milk ingested by the suckling young. The fostering of young from a female of a high mammary tumour incidence strain on to a female of a low mammary tumour incidence strain immediately after birth produces mice which, while genetically susceptible to the action of MTA, develop few mammary tumours in old age because MTA itself is lacking. If the mothers are allowed to suckle their young for only a few hours after birth, the young acquire a sufficient level of infection with virus to develop tumours at the normal rate as they get old. Genetic factors determine susceptibility of mammary tissue to MTA and also determine the ability of a mouse to transmit or propagate MTA. It is also possible that genetic factors control the susceptibility of mammary tissue to hormone. Thus it follows that certain inbred strains of mice, originally selected for this property, have a high incidence rate of mammary tumours among both virgins and multiparous females (e.g., C3H), whilst other strains have very low incidence rates and the tumours may appear only among breeders, as in the A strain. By the use of foster nursing techniques and the transfer of fertilized ova to non-infected strains, various sub-lines of the C3H strain have been derived which have only a low mammary tumour incidence, and this among old mice.

A second important influence on rate of tumour formation is hormonal. Oestrogens control the development of the mammary glands and therefore the tissue in which MTA can produce its action. Thus in some strains virgin females may have an incidence of 5% or less, whilst old breeding females may have an incidence of more than 90%. The development of mammary tumours is also influenced by factors such as underfeeding and overcrowding.

The virus, or mammary tumour agent, is filterable through Seitz and Berkfeld filters, and can be deposited by centrifugation. It survives lyophilization for at least 6 months, but it is killed by glycerol. It survives in the yolk-sac of developing hens eggs, and survives desiccation at room temperature. It does not occur in the ether-soluble fraction of mouse milk or tumour extracts, and is killed in mouse milk at 61°C in 30 min. Neutralizing antibodies

can be produced by injecting virus preparations in rabbits. A mammary tumour agent has not been found in any other species.

Notes on the incidence of mammary tumours in various inbred strains are to be found in Committee on Standardized Nomenclature for Inbred Strains of Mice (1952).

D. POLYOMA VIRUS

While searching for a leukaemia-inducing agent in extracts of leukaemic tissue from AKR mice, Gross (1953) noted that some C3H mice, injected with the leukaemic extracts when new-born, developed tumours of the parotid gland. These were also induced by Stewart who later, in collaboration with Eddy (1955), grew the active agent in tissue culture. On injection into new-born mice, this agent produced a variety of tumours at different sites, and it was therefore called the polyoma virus. The virus (in a latent form) is very widespread among mouse colonies in England and America. It does not appear to give rise to tumours under normal conditions but only when inoculated into new-born animals less than 24 hr old. Subsequently these animals develop a variety of neoplasia. Tumours can also be induced by the same virus in other species, e.g., hamsters and rats; in this respect the virus is unique. Studies of the epidemiology of this disease have been carried out by Brodsky *et al.* (1959), Rowe *et al.* (1962) and Salaman and Rowson (1961). Rowe *et al.* examined mice from different colonies and found that the infection rate (as demonstrated by haemagglutination-inhibition tests) can vary from 0 to 84%. The high rates were found in colonies where there was the greatest risk of environmental contamination, i.e., in laboratories where work with polyoma virus was in progress. They also found that antibody was rare under 3 months of age, and that its frequency increased with age. In infected colonies the highest incidence rates were found among mice in rooms also housing mice inoculated with polyoma virus. They found, in fact, that antibody was distributed by colony rather than by strain. The evidence points to environmental contamination being an important source of cross-infection with this virus in a colony. Inoculated mice excrete virus in saliva, urine and faeces. Salaman demonstrated the slow spread of polyoma virus among naturally infected mice which had been isolated from other possible sources of virus contamination. Salaman's work also demonstrated methods for the establishment of polyoma-free stocks. Thus, he established uninfected lines by weaning litters at 2 weeks of age, isolating these from possible sources of contamination, and subsequently testing these animals regularly for haemagglutination-inhibition antibodies. From these studies it is obvious that measures to prevent the spread of polyoma virus will include the strict isolation of virus-infected animals, the conducting of frequent surveys among breeding stock for haemagglutination-inhibition antibodies and strict control of incoming stock.

A valuable recent review of mouse polyoma infection has been given by Rowe (1961).

E. ONCOLYTIC VIRUSES

An example of a latent viral infection of a different type is that described by Bennette (1960). He isolated a non-pathogenic virus from apparently normal mice which is capable of destroying mouse ascites tumour cells *in vivo*. A similar agent has been described by Nelson and Tarnowski (1960), whose virus appeared to be highly pathogenic for nurslings 1 to 2 days old. These oncolytic viruses are unlikely to be of interest to most users of laboratory mice, but workers in certain cancer fields should know of their existence.

XI. Inherited Diseases, Anomalies and Malformations

As infections of laboratory animals become more and more controlled, so will the group of diseases discussed in this section become of more importance in a practical sense, for many of these conditions are lethal or sublethal at, or even before, birth. It is essential that animal technicians be trained to report the presence of suspected genetic conditions, for they may affect the productivity of breeding colonies. Much more important, however, is the fact that past history has shown the immense value of such conditions for the geneticist and heredopathologist interested in studying the nature of such diseases, and for simple reasons. There is far more known about heredopathology of laboratory animals (and the mouse in particular) than other animals, or man, for any one worker in the course of a year or so can study many generations of inbred mice or rats, and prove conclusively that the condition is indeed genetic in origin. In human beings, as a contrast, no one observer can possibly in his whole lifetime study more than about two generations of any inherited disease, though it might have a history of genetical complication going back for centuries.

In this chapter we can do no more than give a very brief glimpse of the enormous field for observations and research, and indicate highlights. For further information there is available a fascinating short text by Grüneberg (1947) entitled "Animal Genetics and Medicine". A much larger and more extensive review is available in Nachtsheim's chapter in Cohrs *et al.* (1958). Grüneberg's special contribution of some 650 pages on inherited diseases of the nervous system of the mouse alone may indicate the magnitude of this specialized and important field.

Firstly, we should emphasize the difference between an inherited and a congenital disease. A disease seen at birth need not necessarily be inherited, for there are many infections of man and animals which are transmitted from mother to foetus. Similarly, an inherited disease need not always be seen at birth, for many genetic conditions, especially of certain organs, may only become overt when that organ becomes fully functioning. Many genetic

conditions are incompatible with life, and kill at birth or even in the intra-uterine period, so that foetuses are aborted. In mice and rats such abnormalities may never be noticed, for the mothers may eat the dead foetuses. Whenever conditions are suspected of being inherited they should not be glibly ignored, nor should such affected animals be thrown away in the garbage can. They can offer important data on the breeding history of any colony in question, and more significantly the disease can be of supreme importance to geneticists and pathologists who may be interested.

The introduction to a chapter in Innes and Saunders on inherited diseases of the nervous system of all animals should be consulted, even though it is confined to consideration of the nervous system. The main literature is cited, the problems at stake are analysed, and the repercussions such studies may have in related fields such as embryology, teratogenesis and neonatal pathology are all brought out.

Inherited conditions are controlled by genes, which betokens the causal genesis of Grüneberg, but this does not explain the mechanism of their development (formal genesis), and we know a lot more about the former than the latter. However, we know now that environmental influences *in utero*, such as mechanical causes, temperature changes, radiations, oxygen lack, chemical poisons, nutritional deficiencies, hormonal influences, and infections, all may cause disease of the embryo or new-born.

All that has been attempted below is to tabulate a very few of the important well-described inherited diseases of mice and rats. For all further desired information, reference should be made to Grüneberg's text and many of his original papers, to Grüneberg (1956), and also to Nachtsheim.

TABLE II

Inherited Diseases, Anomalies and Malformations of the Rat and Nouse

Organ System	Species	Name	Main Features
Central nervous system	Mouse	Pseudoencephaly (anencephaly, cranio-schisis, acrania)	New-born embryos. Calvarium missing, with herniation of brain, which is literally turned inside out
	Mouse	Absence of corpus callosum	
	Mouse Rat	Hydrocephalus	Large domed head, due to thin membranous skull, shortened face and ballooned-out brain filled with fluid. (Several variants of this condition found due to different genes)
	Rat Mouse	Epilepsy	Seizures elicited by acoustic stimuli
Organ of hearing	Mouse Rat	Waltzing	Whirl or circle about an axis, incessantly for several minutes, some circle to right or left; choreic head movements. Animals stone deaf. Pathology uncertain

TABLE II—contd.

Organ System	Species	Name	Main Pathologic Features
	Mouse	Shakers and jerkers	Probably three different genes; with same triad of signs as waltzers; some shake more than waltzing
	Mouse	Pirouetting	Another choreic disturbance due to a recessive gene; animal whirls in narrow circles pivoted on a front leg
Eye	Mouse	Anophthalmia	Either no eyes at all, or maldeveloped small incomplete eyes
	Rat	Microphthalmia	A small incomplete eye
	Mouse	Rodless retina	Blind mice. Visual purple completely absent and no electrical response to stimulation with light
	Rat	Retinal degeneration with cataract	
	Rat	Congenital cataracts	Small misshapen lens
Endocrine organs	Mouse Rat	Pituitary dwarfism	Normal size at birth, but never grow; have blunt snout, short tail; both sexes sterile. Similar type in the rat
	Mouse	Reduplication of Seminal vesicles	
	Rat	Latent diabetes	Grow more rapidly; reach larger size; tendency to obesity; sterility common
Blood	Rat	Acholuric jaundice	Jaundice at birth with anaemia and splenomegaly
	Mouse	Hypochromic anaemia	
Skeleton	Mouse	Posterior duplication	Four hind legs and two tails with variants
	Mouse	Short tail	Short or nearly completely absent tail, also kinky and fused tail, pig-tail, flexed-tail and screw-tail
	Rat	Taillessness	
	Mouse	Short ear	
	Mouse	Absence of tibia	
	Mouse	Stub	Complex of abnormalities with shortening of trunk, and much vertebral disorder with lordosis, scoliosis and kyphosis
Alimentary tract	Mouse	Hare lip and cleft palate	
	Rat	Non-eruption of teeth	
	Mouse	Stenosis of oesophagus	
	Rat	Umbilical hernia	
Urogenital system	Mouse	A very wide variety of anomalies—one or both kidneys missing; hydronephrosis; absence of ureters	
	Mouse	Imperforate vagina	External orifice of vagina closed
Skin	Mouse	Hypotrichosis	Hairlessness starting around 10-14 days, overgrowth of claws, extensive cystic formation starting from hair follicles and sebaceous glands. Something similar also in rats
Skeletal muscle	Mouse	Dystrophia muscularis	Progressive weakness to ataxia, with atrophy of muscles of hind limbs spreading to axis and forelimbs

XII. Some Important Diseases of Uncertain Aetiology

In retrospect, in view of the prime basic use of laboratory animals for all aspects of medical, veterinary and biological research, it would appear almost unbelievable that in the last 80 years no institute has ever been founded to deal specifically with research into spontaneous diseases of the small laboratory animal. Such is the case. A bird's-eye view of the immense literature now available (see Cohrs *et al.*, 1958) reveals that contributions have mainly come from pathologists, bacteriologists and virologists who have been led into studies by necessity, to clarify some other problem, or by accident, when a disease or condition has been found complicating another under specific study, or through pure curiosity. This section deals with a few important diseases which come into one or other of the above categories, and which because of uncertainty regarding aetiology cannot be placed elsewhere, or it is more convenient to deal with them here.

A. Acute Disease of the Submaxillary and Harderian Glands (Sialo-dacryoadenitis) of Rats with Cytomegaly and No Inclusion Bodies

Salivary-gland virus infection in laboratory animals has been dealt with previously, (p. 67) but it is necessary to recall that in most species the virus causes little inflammatory reaction but produces intranuclear inclusion bodies with cytomegaly in the epithelium of the ducts of the submaxillary gland. The disease then under discussion, from what follows, must come into a different group. The only description in the literature is that by Innes and Stanton (1961), while a paper by Seifert (1960) from Germany dealt with the same condition but in a very extensive histological study on the submaxillary glands of all laboratory animals. Since that date we have heard that the same condition, which is unmistakable, has been found in a number of rat colonies in the U.S.A., and no doubt in time will be found to be wider spread.

The disease causes no mortality and the rats remain active and eat well. The neck is grossly swollen, with the head sunk into the neck, so that the rat looks more like a guinea-pig. The rats show "red tears" and red staining round the eyelids due to porphyrin excretion from the affected Harderian glands. On dissection of the neck massive gelatinous oedema is found, from the mandibular space to the base of the neck, in the midst of which lies the swollen tense submaxillary gland. Pressure on the great veins entering the thorax causes them to be enormously dilated. Histologically, the submaxillary and Harderian glands show a process of acute inflammatory reaction with the lobules spread out by the intense oedema in the loose connective tissue, and in which polymorphonuclear and mononuclear cells, lymphocytes and histocytes are present. In addition, there is a marked hyperplasia of the epithelium of the ducts which is converted into a mosaic of stratified epithelium which

almost occludes the lumen. Some cells are cytomegalic, many mitotic figures are present, but there are no inclusion bodies in the nuclei or cytoplasm of these epithelial cells.

No clue regarding aetiology was obtained by Innes and Stanton or by Seifert. The inflammatory reaction might betoken an infection, while the duct changes make comparison with vitamin A deficiency necessary. The latter can be excluded, for the keratinization of epithelium in the latter condition is widespread throughout many organs in the body.

B. A HAEMORRHAGIC DIATHESIS WITH MYOCARDITIS IN CERTAIN STRAINS OF MICE

The story begins with observations by Angevine and Furth in 1943. They recorded a fatal disease of well-developed adult male mice producing sudden death and associated with myocarditis, exsanguinating haemorrhage into the pleural and pericardial cavities, and invariably accompanied by testicular haemorrhage. It was found most commonly between 10 and 19 months of age. Epidemiological observations and transmission experiments and cultural studies failed to prove the condition was infectious.

Recently, the same or similar disease has been under intensive study by workers in the Jackson Memorial Laboratory, Bar Harbor, U.S.A. (see Meier, Allen and Hoag, 1962). If the disease is not the same aetiologically, it has an exact counterpart in the main pathological abnormalities seen by the naked eye and microscopically, i.e., the haemorrhage in pleural and pericardial cavities and testes, and the acute myocarditis. Considerable other work was done by Meier, all in an attempt to resolve the haematological changes. Their laboratory work on clotting tests, component assays and substitution analysis and electrophoresis is impressive in the extent and scope of what was done. The result showed that liver dysfunction was the most important clinical consequence. Basically, the mice suffered from single and multiple prothrombin deficiency. The disease occurred only in adult males in seven different inbred strains of mice, and very obviously is of the utmost practical importance, for they mention that nearly 1,000 mice died. Such mortality indicates that the condition could wreck any special breeding programme.

One other important item was uncovered by Meier *et al.* in their attempts to identify aetiology. They were able to show that the disease could be at least precipitated by ethylene glycol, and that the latter was produced during the process of sterilization by the ethylene oxide-carbon dioxide process of the wood shavings used by them for bedding.

Concurrently with the above work, Innes and Borner (unpublished work) have encountered and studied a replica of the haemorrhagic diathesis in their "disease-free" colony of mice. There was the massive haemorrhage with its predilection for pleural and pericardial cavities and testes, the acute myo-

carditis, and also its occurrence almost exclusively in adult male breeders. This colony was started by stock which originally had been caesarean-derived and established under good isolation, breeding being done by monogamous pairings. The first case did not occur until about 4 months after the colony was started, and thereafter at irregular intervals. Mortality at all ages from birth on in this colony and from all causes has been very low—viz., 6·8%. In the face of all evidence we were inclined to consider the condition as a sex-linked genetic one, due to a recessive factor, until the appearance of the papers by Meier *et al.*, and we still believe that there may be (must be) some genetic background. The ethylene glycol aspect of the onset of the condition reported by Meier *et al.* cannot enter into the aetiological picture of our (Innes and Borner) mouse condition. Firstly, dry corn cob (Sanicel), which is sterilized by high dry heat, was used for bedding and enquiries from the food pellet manufacturer revealed they did not use the ethylene oxide method of sterilization on their pellets or any content of pellet. At the time of writing no hematological work has been done and all attempts to identify any toxic factor which might precipitate a haemorrhagic syndrome, as in Meier's mice, have failed. What has not been uncovered by any of the above investigators is the origin of the haemorrhage, for such massive bleeding can only arise by vascular rupture of heart, large veins or arteries—in other words we still do not know if there is any concurrent arterial or venous disease.

C. NECROTIZING ARTERITIS IN STRAIN BL/DE MICE

Deringer reported on this condition in a highly inbred strain of mice, the foundation of which dates back to 1921. The original observations sprang from an examination of ovaries in which the corpora lutea were found to be heavily infiltrated with amyloid material in female mice 10-13 months of age. Later, it was found that arteries and arterioles in the hilum of the ovary, in the perirenal adipose tissue, in vessels of the adrenal cortex and in uterine and mesenteric vessels all showed the same change. The vascular lesion was essentially that of thickness with deposition of an amorphous substance in the walls (intima and media), and marked infiltration of the adventitia and extra-adventitial tissue with lymphocytes and plasma cells. No cause was ascertained. Other workers have described lesions in arteries of mice, regarded as a periarteritis nodosa affecting the vessels of subcutaneous adipose tissue, ovary, stomach wall and salivary glands.

D. PERIARTERITIS IN RATS

Skold (1959) in a follow-up of the paper by Deringer described a condition in rats. He stressed that the term was used to avoid any true connotation with periarteritis nodosa in human beings. He found 47 out of 108 rats with the lesions described. Periarteritis means inflammation of the entire circumference of arteries. To the naked eye there are dark beads along the vessels,

particularly in the vascular bed of the mesentery and sometimes only along pancreatic arteries.

The nodules may develop into aneurysms and rupture with massive haemorrhage. The thickened arteries feel like wire. Histologically, there is necrotizing involvement of intima, media and adventitia, with eosinophilic infiltration in and round the vessels. There may be blocking of the lumen by thrombosis, while narrowing of the lumen leads to reparative fibrosis and scarring of the affected wall. Such arterial changes may be partly responsible for the very common chronic nephritis, with the obviously (to the naked eye) contracted kidney, seen so often in aged rats (and mice).

E. PULMONARY PHLEBITIS AND MYOCARDITIS IN MICE ASSOCIATED WITH RICKETTSIA-LIKE BODIES IN POLYMORPHONUCLEAR LEUCOCYTES

From what follows, it is not out of place here to state on record that the late Dr. Alwin Pappenheimer, after a lifetime in medical pathology which he graced with great distinction, in his retirement probably found more new diseases of laboratory animals than any past or existing worker. There remains to be said that in the lungs of rats and mice the histological structure of the intrapulmonary veins is peculiar in that they carry in their walls a layer of true cardiac muscle. This feature was alluded to by Innes *et al.* in their paper on chronic murine pneumonia, but it is hardly ever mentioned in English literature. Others besides Dr. Pappenheimer have mistaken veins for arteries in the rodent lung, so in his original paper for the title of *Pulmonary Arteritis* we have substituted the word phlebitis. It makes no difference to the importance of the paper. Clearly, it might have some connection with the haemorrhagic condition described earlier, but only in so far as the occurrence of an acute myocarditis is concerned.

The disease was studied largely in mice during blind passages of tissue emulsions from a moribund mouse. The heart showed acute interstitial myocarditis, affecting atria and ventricles, and there was no vascular thrombosis. In contrast to the myocarditis produced by various strains of Coxsackie virus, there was never any marked degeneration or necrosis of cardiac muscle. The distinctive feature was, however, the involvement of the cardiac muscle (within the lungs of mice and rats) which surrounds the intrapulmonary veins. In other words there was, in addition to true myocarditis, a paradox of an intrapulmonary myocarditis, which feature seems to have escaped all subsequent observers. No exact knowledge was ever obtained on the aetiology of this extraordinary condition, perhaps because of Dr. Pappenheimer's untimely decease. He observed and commented upon a clue to possible aetiology in the presence of innumerable minute coccobacillary bodies in the cytoplasm of polymorphonuclear leucocytes. The position has not been altered by any further publication, and to our knowledge no one else has ever recorded the occurrence of this disease of mice.

F. RINGTAIL IN RATS AND MICE

Ringtail is a condition found occasionally in young rats, and sometimes in older rats and C 57 mice. Affected animals show annular constrictions of the tail, with oedema, necrosis and eventual amputation. The condition is associated with low temperature and low humidity but may also be influenced by factors such as fat metabolism, essential fatty acids and skin permeability. Flynn (1959) gives a good account of this disease. Possibly the lesions have a vascular basis; in one rat examined by (J.R.M.I.) an organising phlebothrombosis has been found above the site of the necrosis.

XIII. Special Pathology of Organ Systems

This aspect of diseases of mice and rats is of immense importance to the experimentalist using these animals and particularly when they are being kept for a year or more under observation until death, e.g., longevity studies, chronic toxicity tests with new drugs, tumour work, effects of irradiation on survival and so on. It then behoves the investigator to know what lesions or diseases (which are clinically silent) occur in all the organs of the body, and which are outside the category of all those already dealt with in previous sections, i.e., specific bacterial, viral, parasitic and inherited conditions. These tissue changes can be caused by advancing age, nutritional imbalance, or hormonal influences or they can come into the type known as organic disease of no known specific aetiology. Some of these can be contributory to ill-health and precipitate death, so that it occurs earlier than it otherwise might from whatever experimental procedure had been adopted. The lesions may be found incidentally, when routine histological surveys are being undertaken, e.g., in chronic toxicity work with chemical compounds. A knowledge of their nature and importance becomes of significance to avoid false conclusions being drawn that they were produced by the agent under test. The first volume of Cohrs *et al.* is very largely concerned with this topic and contains some 800 pages and thousands of references. Another invaluable treatise is that by Cottier (1961), who in an experimental irradiation study in mice, examined histologically every tissue in the body of hundreds of non-irradiated mice allowed to die natural deaths as a control to a study of the effects of experimental irradiation. In this present book, which is intended for quite a different audience (and apart from space considerations), the subject can hardly be given any lengthy detailed consideration.

Nevertheless, it was thought that an abbreviated list of common lesions found in the different organ systems, particularly of mature and old mice and rats, might be of general interest. One word of warning might be uttered, namely that what might be found very commonly in one strain of inbred mice or rats may not apply to another strain, e.g., the frequent incidence of pituitary tumours in Sprague Dawley rats is not equalled in any other strain. In

the end, individual observers will simply learn by experience, and an acquisition of knowledge of pathology takes years.

1. Cardiovascular system. Cartilaginous changes in papillary muscles; endocarditis and myocarditis; arteriosclerosis of main trunks; medial calcification; periarteritis nodosa.

2. Respiratory system. Malformations: foreign bodies in nasal cavities; fragments of bone in lungs; adenomatosis in adult animals; medial calcification of pulmonary arteries.

3. Digestive system. Malformations of all kinds affecting mouth, jaw and teeth; gastric ulcers and erosions: benign and malignant tumours of stomach: senile atrophy of pancreas; lipomatous atrophy of pancreas; inclusion bodies in liver cells of mice.

4. Blood and blood-forming organs. Various anaemias; leukaemia; thymoma; myelosis; chlorosis (see under specific viral infections affecting lymph nodes).

5. Urogenital system. A large number of malformations affecting ureters and genital organs; lipid, glycogen and calcareous depositions in kidneys; chronic nephritis in advancing age in mice and rats with and without concomitant vascular changes; testicular atrophy; endometritis; ovarian cysts and abscesses; tumours of ovaries, kidneys, testes, uterus and mammary glands.

6. Endocrine glands. Pituitary tumours, mostly chromophobe adenoma in certain strains of rats: pineal cysts: thymic cysts and tumours: accessory adrenals and malformations: ceroid (brown, amyloid and lipid) degenerations of adrenal glands in mice.

7. Skeletal system. Degenerative arthritis: anomalies and malformations of bones; fractures: atrophy of muscles; parasitic infestations of muscle.

8. Nervous system. A wide variety of malformations affecting all parts, particular parts, or the whole brain; tumours; parasitic infections such as encephalitozoön; degenerative conditions of unknown cause.

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Chapter 4

Specific Pathogen-free Animals

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I. Introduction

A. Definition of SPF

For the term Specific Pathogen-Free to be biologically meaningful, the specific pathogen or pathogens must be stated and the clinical and laboratory methods employed for their detection should be described. This is the more important since virtually no one uses the term as suggested here and, moreover, most biological scientists and animal breeders seem to agree that it is not the most desirable nomenclature. However, as a result of the habitual use of the term for several years, coupled with the convenience of using the abbreviation SPF, it appears that for the time being, at least, we must accept it.

There are many workers in the field of freeing animals of their common diseases who are in agreement that SPF animals are those initially delivered by caesarean section and subsequently maintained in some type of barrier system. The author is one of the proponents of this definition.

B. Efforts to free colonies of disease

Through the years producers and users of laboratory animals have suffered serious losses of time and money resulting from disease and mortality. Good husbandry alone has failed to remove and eliminate the common diseases and parasitic infestations. In many instances testing and eradication programmes merely reduced overt disease while the latent and inapparent infections remained a constant threat to both producer and research investigator.

1. Testing and chemotherapy

In an effort to combat this serious threat to biological research, every known broad spectrum antibiotic was screened against all the common rodent diseases. The experiences of both producers and research workers have demonstrated that commonly found pathogens and parasites of small laboratory rodents, with few exceptions, can be only reduced by chemotherapy. Total elimination has been successful in a very few specific situations.

Freeing animal colonies of a single specific parasitic, bacterial (Wickert *et al.*, 1958) and viral infection (Briody, 1959) has been successfully accomplished by tedious testing and eradication programmes. Usually when sizeable populations are involved this procedure takes a great deal of time and in the final analysis involves restarting the colony from tested or treated nucleus stocks.

There are conventional colonies where some specific diseases and parasitic infestations have been reported controlled by testing and eradication, chemotherapy or both; salmonellosis (Slanetz, 1948, Rabstein, 1958), labarynthitis (Grice *et al.*, 1956), epizootic diarrhoea of infant mice (Kraft, 1958), bartonellosis, oxyurid infestation (Williams and Habermann, 1957) and external parasites (Flynn, 1955). It is likely that only a few of these difficulties, at best,

are controlled in a single colony. In most situations, if the colony is large and the morbidity rate high, the total elimination of even a single entity is an accomplishment worthy of note. There are also colonies thought to be healthy because of a low percentage of clinical signs, but under experimental stress latent infections become apparent and mortality rises sharply.

2. The caesarean concept

Since it appeared unlikely that colonies could be freed of most of the common pathogens and parasites by currently available diagnostic tools and chemotherapeutic agents, a different approach was sought. The ideal situation would be one in which all of the common diseases could be eliminated simultaneously.

How could this be accomplished? In germ-free life studies, or gnotobiotics, animals are not only freed of pathogenic organisms but also from all micro-organisms. The procedure employed in producing gnotobiotes, or germ-free animals, is the aseptic removal of the foetuses from a gravid female by caesarean section, and their introduction and maintenance in a sterile environment. The foetal placenta acts as a very efficient filter and, as far as is known, protects the foetuses from all the bacterial and most of the viral agents carried by the mother. Only a few parasitic forms are known to gain access to the foetuses by placental penetration in their larval stage. Thus, utilizing germ-free technology, caesarean delivery of infected colonies can initially free the colony of all the common pathogens and parasites in one all-inclusive step (Gustafsson, 1948; Reyniers, 1957). Early pioneers in this field (Henthorne and Veenstra, 1957; Nelson, 1951, and 1957; Innes *et al.*, 1957) proved this in small institutional colonies. Their work was later carried on by commercial breeders (Foster, 1957) so that today there are many caesarean originated animal colonies of rats and mice being reared with varying types of environmental control.

II. Terminology

Semantic problems can be overcome at the outset by briefly mentioning other commonly used terms.

Other than SPF, some of the terms most frequently used by producers and investigators are "clean animals", "disease free", "pathogen free", "caesarean started", "free of commonly occurring pathogens and parasites" and "caesarean derived". All these terms are synonymously and interchangeably used at scientific meetings. However, in almost every instance they mean that the initial colony was started by caesarean section and the pups either hand fed or foster suckled on gnotobiotes. The nucleus and production colonies are subsequently reared in specially designed quarantine cages usually operating under a barrier system, and everything entering the barrier is decontaminated, pasteurized or sterilized.

A. TERMS COMMONLY USED

1. Barrier system

This term refers to a method of colony operation where animals are maintained inside a physical barrier where all materials that enter are sterilized, sanitized or pasteurized, depending on the physical characteristics of the material.

2. Germ free

Animals free of all detectable micro-organisms (Reyniers *et al.*, 1949).

3. Gnotobiote

Gnotobiote means "known flora". This term is currently being adopted and used instead of germ-free, since it is more accurate (Reyniers *et al.*, 1949).

4. Plastic isolator

A flexible film plastic chamber developed by Trexler and Reynolds (1957) and Trexler (1958) at the University of Notre Dame for the rearing of gnotobiotes, and also for performing sterile surgery. In the latter it may be referred to as a surgical isolator.

5. Clean foster mother

A lactating female from another SPF colony, or preferably from a gnotobiote colony having recently littered.

6. Entry locks

A series of small rooms used by personnel to change clothing and scrub-up prior to entering the barrier. Basic design can vary from a single large room with partial partitions to individually sealed rooms under different pressures and preferably connected by electrically interlocking doors.

7. Exit hatches

Specially designed exits from which animals and materials can safely be discharged through the barrier and from the building without the entry of vermin or unfiltered air.

8. Dunk tank

A tank, preferably of a non-corrosive material such as stainless steel, containing a germicidal liquid and projecting through the barrier into the clean area. Materials are submerged and passed beneath a vertical partition which separates the contaminated and clean sides. Materials which have previously been sterilized in a plastic membrane are usually passed through this type of tank.

III. The Caesarean Operation

Probably the most important step in the creation and operation of SPF animal colonies is the initial caesarean operation. If this early phase permits pathogens to contaminate the foetuses, all future work will necessarily compound this early error. It is, therefore, extremely important to perfect the caesarean technique and be aware of the possible pitfalls.

A. GENERAL CONSIDERATIONS

1. Proper timing

The first consideration is to perform the caesarean section as close to term as is possible. In fact, the ideal situation is to utilize observed or timed mating techniques. When newly derived pups are properly timed, the survival rate is greatly increased. When observed matings are for some reason impossible, careful palpation and experience can act as a reasonable substitute. Experience has proved that in a high percentage of cases litters are naturally born between 11 p.m. and 6 a.m., making caesarean surgery awkward and tedious.

2. Delaying normal parturition

Progesterone injections daily from the 17th day will, in a high percentage of cases, delay normal parturition so that daytime surgery can be undertaken, virtually eliminating all-night vigils.

3. Premonitory signs

Some feel that a blood spot or the actual natural delivery of one pup signals the ideal time for surgery. This, however, requires constant observation and the necessity of losing at least one pup. Proponents of this technique argue that it is better to lose one or two pups than risk premature surgery. Actually, premature surgery can result in wasted surgery time and valuable animals. Whichever method is used, extreme care at this stage is essential.

4. Foster suckling versus hand feeding

Caesarean delivered animals can either be hand fed or foster suckled on gnotobiotes. With the availability of gnotobiotes from the Lobund Institute and some Government institutions, most individuals desirous of starting SPF colonies may avail themselves of foster nursing stock rather than resort to the highly skilled procedure of hand feeding round the clock for 17-18 days with expected losses (Pleasants, 1959). Timing surgery to coincide with available lactating females is therefore the second essential phase in creating a nucleus stock.

B. SURGICAL TECHNIQUE

The actual surgical procedure is usually conducted in one of two ways.

1. Totally within sterile chamber

The first method, utilized and developed by workers (Reyniers *et al.*, 1946) in germ-free, or gnotobiote, research, involves elevating the gravid, depilated, sacrificed female to a taut plastic membrane in the base of a surgical isolator. A full 10-15 min is available after respiration stops in the sacrificed mother. The technician, with his arms in surgical sleeves and gloves, performs a hysterectomy from within the sterile chamber lifting the intact gravid uterus through the surgical membrane in the floor of the plastic isolator into the chamber proper.

2. Hysterectomy then sterile chamber

The second method, utilized primarily by workers in SPF animals (Foster, 1959a), consists of performing an aseptic hysterectomy on a depilated gravid female, killed by cervical fracture, then introducing the entire uterus and contents into the surgical, sterile isolator *via* a germicidal liquid trap.

Caution must be taken not to rupture the uterus and foetal membranes, for once the foetal envelope is broken sterility is lost. Also, care should be exercised not to cut, tear or break the intestinal wall accidentally. Faecal contents that may carry parasitic ova and pathogenic micro-organisms may exude through the break in the intestinal wall and cover the exterior uterine wall. The brief exposure to a germicide may not be sufficient to kill bacterial organisms and most likely will be totally ineffective against parasitic ova.

3. Removing pups from foetal membranes

Within the isolator, in either technique, the foetuses are removed from their uterine attachment and then individually freed of their foetal membranes. After cauterizing or clamping and twisting the umbilical cord the newly delivered pups are massaged briefly to stimulate normal respiration. Their bluish-grey colour quickly gives way to the bright healthy pink of oxygenated blood. If respiration is slow in starting and frequent massage appears necessary to maintain breathing, the prognosis for survival is poor. On the other hand, when the timing is correct, the foetuses seem anxious to free themselves of the uterine and foetal membrane imprisonment, and the simple stretching and severence of the umbilical cord is sufficient stimulation for respiration.

4. Discharging pups from sterile system

In some instances when multiple caesareans are performed it is wise to complete all surgery before discharging the pups by either the germicidal trap or breaking into the system. When surgery is completed the pups are sexed and counted and family units of 10 females and 2 males are placed into surgical towels and then into sterile plastic bags. The open ends of the bag are twisted, folded and tied with sterilized cord or rubber bands. A second

plastic bag is recommended as the integrity of a single plastic bag cannot be assured. The pups can then be transferred from the surgical isolator to a nearby area or even to a remote location dependent on the size of the bag and the amount of available air locked within the transfer plastic bag. If the pups are to be hand-fed they may actually remain in the surgical isolator or may be transferred to a rearing isolator.

C. THE FOSTER MOTHER

1. Selection of mother and preparing nest

If the pups are to be foster nursed by gnotobiotes, some special considerations are necessary. The foster mother is best left in the same cage with the nesting material in which her own litter was born. Her own offspring are separated from her preferably 48-72 h after parturition. Trial and error will best determine the optimal separation time for the particular strain in question to insure the best acceptance period and optimum lactation.

It is entirely possible that strain differences will be a factor; however, rats as a general rule accept foster pups best shortly after parturition. In the case of mice this is not as critical. Some have reported in mice that any time during the three-week suckling period is satisfactory.

2. Temporary removal of mother from nest

After the naturally born offspring have been taken away, the mother is temporarily moved to a clean cage with fresh bedding. It is at this point that the caesarean delivered pups are introduced into the motherless nest. In this way the surgically delivered pups are given the opportunity to take on the scent of the foster mother, thus minimizing cannibalism resulting from a foreign odour. Some have, in addition, resorted to placing highly aromatic substances on the foster mother's nose when introducing the pups into the nest. This momentarily desensitizes the olfactory sense of the mother, thereby decreasing the foreign odour problem. However, careful manipulation and planning will circumvent the cannibalism factor.

3. Return of mother to her nest

After the pups have been neatly tucked into her nest and covered over with her own bedding, the mother can be returned to her cage. Precaution must be taken that hands are washed thoroughly and freed from odours. After 20-30 min the cage is carefully observed to see whether the mother has accepted her new family. If she is nesting over them, one can be reasonably assured that no difficulty will ensue. If, however, she is paying no attention to them, she must be further checked 30 min later. After a total elapsed time of 1 h, if the new pups are still being ignored, a new mother is tried following the introductory procedure previously described.

4. The critical period

Usually by this time a compatible family unit is found. The first 12-24 h are critical. If the pups survive this period and are showing a milk line, the prognosis is good. If, however, they have not suckled during the first 12-24 h it is unlikely that they will survive at all. Premature pups invariably are dead or die at this stage and are sometimes eaten by the mother so that no trace is to be found. Again, let it be emphasized that properly timed pregnancies produce viable pups in nearly every instance. It is mainly when pups are delivered prematurely that failure results. With experience the trained eye can almost invariably predict the success or failure of the caesarean by the size and appearance of the new-born.

5. Colony cross-section

In order to ensure a reasonably broad genetic sampling of a random colony, twenty-five to fifty caesareans should be performed. This insures a cross-section of the strain's genetic characteristics and minimizes the possibility of perpetuating specific, undesirable genetic characteristics. In the case of highly inbred lines of 20-plus generations, the number of caesareans can be as few as one or two since at this stage the genetic characteristics are considered homozygous.

6. Bacteriological check of mother

The cautious worker, before introducing newly derived offspring into a nucleus area, performs careful bacteriological examination of the entire genital tract. Some have reported finding pleuro-pneumonia-like organisms in the genital tract of small rodents which might invalidate relying exclusively on the placenta, since contamination may occur initially at the time of implantation. Granted, this possibility is the exception rather than the rule and as new information is developed it is best to take every precaution by pursuing every avenue of possible infection. Other than intra-uterine infection or careless technique, present information has not discovered any loopholes in the caesarean section principle for deriving clean or gnotobiotic stock.

IV. The Nucleus Colony

The next phase in developing SPF colonies is the propagation of the caesarean litters into a nucleus colony of sufficient size eventually to stock a production unit. It is a good practice to maintain the nucleus colony with more safeguards than the future production unit, since the nucleus stock may be called upon from time to time for stocking new areas or restocking old areas. Since caesarean delivery eliminates rodent pathogens and parasites, protecting the nucleus from human organisms would ensure that the nucleus colony has a lower microbial flora than the production unit which is routinely exposed to the technician.

A. TECHNICIAN WEARS PLASTIC SUIT

Practical proven plastic systems originally engineered by Trexler and his group have been utilized with minor modification. Complete enclosure in a plastic suit with forced ventilation seems to work quite efficiently (Foster, 1961). Ventilation may be accomplished by a $1\frac{1}{2}$ in plastic tube intake, with an exhaust provided by a small portable blower system which drives exhaled air through a filter trap before being vented into the room. An alternative system places the blower outside the room so that exhaled air is driven out of the nucleus room directly. The latter system requires a long, flexible, lightweight pressure hose, while the former allows the self-contained exhaust system to accompany the technician, followed only by a power cord. Both systems accomplish the same end, namely preventing human exhalations and contaminants from contacting the nucleus colony.

B. DECONTAMINATING EVERYTHING ENTERING BARRIER

Another added safety factor is the sterilization of water. Other than protecting the animals from the human respiratory tract and sterilizing the water, the maintenance of the nucleus is similar to the barrier system operation of the production colony. In the barrier system everything entering the clean areas is sterilized except the food which is pasteurized rather than autoclaved to minimize nutrient destruction. The air entering the animal area passes through special filter banks capable of filtering fractional micron particles. In the production barrier system personnel enter by a series of entry locks. In the nucleus, after being sealed into the plastic suit, the technician stands in a small chamber which is saturated with a germicidal mist for a sufficient length of time to destroy pathogens. This affords complete protection against the entry of micro-organisms on the technician, since he is sealed in plastic and the surface of the plastic is sterilized with a germicide.

C. LABORATORY CONTROL

Routine necropsies, histopathology and bacteriology are performed on a random sampling of the nucleus with particular emphasis on discarded breeders. Careful examination for the common pathogens and parasites should continue indefinitely at regular intervals, for this is the only true method of maintaining current information on the microbial flora of the animals. Evidence of any of the common pathogens or parasites indicates a weakness in the system and warrants starting anew from the caesarean operation. However, with careful technique and planning, contamination at this stage is unlikely since maximum barrier security is practised and personnel contact is eliminated. When large numbers are reached and exhaustive testing of the animals proves negative, preparation for large-scale production is undertaken.

V. Planning the Production Colony Building

A. General considerations

Some basic goals must be set and met in order to expand the nucleus stock into a large production colony. Since flying and crawling insects and wild rodents are sources of contamination, the production facility should be designed to preclude their entry. Designing the structure without windows and with only the necessary number of doors is important. Also, wherever an exit is needed from the building this should be so designed to prohibit vermin and backdraughts of unfiltered air. The basic structure is best fabricated from masonry materials which are impervious, do not harbour vermin and are fire-resistant as well. Other general considerations are entry locks for personnel, year-round heating, ventilating, humidifying, dehumidifying, cooling and filtration systems, facilities for sterilization, sanitization, and pasteurization of supplies, safety exits for animals, safe method for removing excrement, and emergency power in the event of power failure (Foster, 1959b, 1961).

B. Floor plans

1. Small independent production rooms

Before going into construction detail a floor plan must be developed. The trend in recent years is towards many independently operating breeding rooms, as opposed to the single large production room. This affords insurance against loss of an entire production facility in the event of the introduction of disease. Common agreement has not been reached on the optimal production room size for rats and mice, since current opinion seems to be based on how many technicians will service the room rather than the number of animals to be produced from a given area.

a. Some advocate singly operated production rooms, since this system instils a strong sense of responsibility in the individual, offers a competitive spirit to out-produce fellow-workers in other areas and theoretically reduces contamination possibilities.

b. The opposite view states that many people dislike working alone, particularly in a windowless structure. Also, it is inevitable that another technician will, through necessity, enter the room during vacation periods and in the case of illness. Some also feel that a small room designed for the single technician is too costly, since basic utilities have to be brought to each area at maximum cost when for a minimal increase in equipment size and cost, with little additional labour, the area size could be doubled or trebled. Therefore, rooms have been designed to accommodate one, two, three or more persons, dependent on budgetary limitations and personal opinion.

2. Holding or stock area

When the size and number of production rooms is decided upon, holding or stock facilities must be planned. Producers of mice prefer that the stock

area be designed to accommodate 2 weeks' production, since most mice used are under 25 g, or 2-2½ weeks post-weaning. In rats, where common utilization is as high as 60 days post-weaning, the ratio should be greater since the required holding period for rats is longer. If funds are available, it is better to have more space than required so that generous housing allowances and latitude are provided for in peak holding periods.

For the most efficient utilization of space, production areas can share a common wall with the holding area, permitting positive pressure chutes from the production rooms to the stock area for the daily transport of newly weaned animals. With this type of arrangement there is maximum utilization of floor space by the elimination or minimization of communication corridors.

3. Minimizing corridor areas

There are specific considerations to be engineered in utilizing the concept of direct pass-throughs from breed to stock areas. If corridors are eliminated there must be other means provided for the transportation of feed and bedding, and the removal of excrement. The usual method of material transport is from a central storage area to the individual animal areas *via* common hallways.

Isolation of individual production areas is somewhat diluted by personnel servicing the areas with supplies through the same corridors used for the movement of animals from each breeding area to the stock area. If the corridor space is minimized or eliminated, the gain in space can be utilized for additional production or holding areas.

In order to accomplish these aims, provision must be made for the safe entry and exit of personnel in and out of all the areas independently.

4. Maximum isolation and independence

This requires separate entrance locks, lavatories and eating facilities for each area. In effect, the end-result is the construction of independent animal areas with separate entrances and exits still under the same roof, on a common foundation, sharing utilities and sewage disposal facilities. The ultimate and the most costly is a series of small buildings completely independent of each other and individually supplied with the basic services. Cost almost prohibits such construction for most privately owned commercial profit-making organizations and seems possible only by endowed or supported research institutions. Actually, the single foundation and roof concept with non-communicating animal rooms, other than passages or chutes for animals, appears sound and realistic, and it works.

C. MATERIAL TRANSPORT

It is now necessary to consider the transport of feed, bedding and excrement. There are some producers who have partly or totally automatic

facilities, thus solving the material transport problem. Some have gone beyond the transport of feed to the animal room and deliver it directly to each individual cage.

1. Feed and bedding conveyance

Feed and bedding can be successfully transported either mechanically or pneumatically, depending on the individual situation. Grain elevators and belts handle large volumes of material but are initially very costly and require a fair amount of maintenance. Pneumatic conveyance of supplies is more desirable since initially the cost of installation is less, and in practice, even though the tube size limits the volume, it is cleaner and requires minimal maintenance.

2. Excrement transport

Again, without central passageways excrement and soiled bedding must be transported directly from each separate area. This may be accomplished by belts, automatic flushing systems, screw-type conveyors in a pit beneath the concrete floor, or by a system of pneumatic tubes under vacuum.

D. CAGE AND BOTTLE SANITIZATION

One further major controversy exists in the design and planning of a structure of this type. Are the cages, bottles and feeders to be washed routinely? If so, is this accomplished within or outside the barrier? There are some who argue that weekly cage washing and sanitization of equipment is fundamental and a dictate of good husbandry practice. They argue that it is essential in controlling infectious agents by reducing concentrations and numbers. This is undoubtedly true, but others have attuned their thinking to the field of gnotobiotics, where total reliance is placed on the barrier and related technology.

In a given animal area, within a barrier, isolation is maintained from adjoining animal areas. But, of course, there is no separation or quarantine within the individual area. Therefore, one may assume with some assurance that a uniform flora and microbial equilibrium is developed *via* air and personnel transfer, and that animals from one side of the room demonstrate the same flora as those from the opposite side. Actually, if this premise is true it represents an advantage by way of uniformity within the research animal. Tests have demonstrated that over a period of time animals within a barrier offer more uniformity of flora than animals maintained otherwise. Thus, cages and bottles can either be removed, or maintained in the barrier, for washing, or total reliance on the barrier can forgo washing entirely.

In general, therefore, a basic floor plan is conceived as a series of non-communicating production areas with separate entrance locks, whose

proximity to the stock area enables weaned stock to be passed through a positive pressure chute to the holding area. Feed and bedding are transported from the sterilizing area to the animal quarters by some type of automatic or semi-automatic conveyance system. Soiled bedding and excrement are removed from the animal areas either mechanically or pneumatically.

VI. Construction of Building

A. VERMIN-PROOF MATERIALS

After consideration has been given to the room arrangement, and a plan evolved, actual construction can commence. In general, vermin-proof materials should be used with additional consideration to fire resistance. The ideal structure, according to fire insurance underwriters, is an all-concrete structure, even though common belief has it that steel joists and roof deck is the ideal. In reality, steel will bend and cave in under intense heat, whereas concrete will not. The shell of the building, therefore, would best be constructed with a reinforced concrete foundation and either cinder blocks, cement blocks, or precast concrete walls and ceilings without an air space above. The roof, ideally, should be concrete, of precast slabs such as are commonly used in many parts of the U.S.A. The initial cost is greater than steel span or wood trusses, but the annual saving in insurance amortizes this difference quite readily. If all-concrete construction is followed, the addition of a concrete floor creates a total concrete fire-resistant envelope about each animal area, ceilings, walls and floors.

B. INSULATION

Insulation of the wall and ceiling can be provided in various ways. One should bear in mind that many qualities are sought in an insulating material.

1. Insulating material

First, insulating material should provide a good vapour barrier, since relative humidity within the areas must be maintained. Ideally, insulating material should also be fire-resistant, vermin-proof, inert, and be able to maintain its original qualities year after year. Most insulating materials do not fulfil the above prerequisites, and in addition require some form of studding to hold them in place as well as to provide support for the interior finish.

2. Installation of insulation

There is at least one rigid insulation which is inert, fire-resistant, provides an excellent vapour barrier, is vermin-proof and can be attached directly to a masonry wall by means of an adhesive. It is applied in large blocks and can have incorporated within its installation steel clips which hold wire lathing in place for a plaster-finished wall. Foamglas, manufactured in the U.S.A. by

the Pittsburgh Corning Corporation, is somewhat more costly than more commonly used materials but is almost ideal for structures requiring critical environmental control. When this insulation is applied to the walls, a base coat of plaster is applied to the wire lathing covering it followed by a skimming coat of cement plaster finish.

C. INTERIOR FINISH

If precast concrete slabs are utilized for the ceiling, the joints are grouted with a flexible caulking material which expands and contracts with environmental changes creating an unbroken, smooth surface.

The walls and ceilings can be finished with a variety of modern finishes varying from the universally used water and oil base paints to lacquers and epoxy resins. It is wise to use a finish which will have a long life and can be retouched when the occasion arises. Materials having pungent odours should be avoided as they present difficulties when animals are present. This would make retouching, patching or repainting almost impossible without complete breakdown of the unit. Ceilings require less surface maintenance and can be finished with a sprayed-on material. In windowless structures, light pleasing colours help to minimize the feeling of claustrophobia. In many institutions white is still the most popular finish.

D. FLOORS

Floors present a very special problem and have a variety of approaches. It is difficult to find two people who can agree on a floor finish, since the stresses placed on floors vary with caging, foot attire and usage. Some plastic paints form a firm bond with new concrete surfaces, in particular, and can be removed only by actually chipping the concrete. If aesthetics can be overlooked, an unpainted concrete floor with a surface hardener and sealer may offer the most practical solution to the maintenance problem which inevitably arises. Colour pigment can be impregnated into concrete, but evenness of colour is difficult to achieve.

Tiles in a variety of materials, ceramic, plastic and linoleum, are occasionally used, sometimes with good results. Because of the spacing that exists between tiles, however, cleaning and maintenance may be difficult and cleaning water seeping underneath may loosen and raise them. Ceramic tiles are also brittle and may be subject to breakage.

E. LIGHTING

Lighting engineers seem partial to rapid or instant-start, 8-ft continuous, fluorescent strip lighting. Surface mounted, the strips are readily accessible, emit even illumination and have a long tube life. With concrete ceilings everything, necessarily, must be surface mounted. However, because it is essential to be able to give an area a thorough clean-out, nothing must be

concealed and inaccessible. Warm colours are sought in fluorescent lighting, since some of the so-called cool white colours impart a look of anaemia to the animals. The warm series, which vary in designation with the manufacturer, have the closest resemblance to incandescent lighting, which imparts the normal pink to the ears and extremities of rats (but not so much to mice, which are naturally less pink). Lumen output per watt of electricity consumed is most efficient in 8-ft fluorescent fixtures.

VII. Environmental Control

Under this general heading are included filtration, ventilation, heating, cooling, humidification and dehumidification. In considering this total subject of environmental control it is well to consider each integral phase separately or in combination.

A. AIR FILTRATION

Air filtration can be accomplished by air washing, electrostatic precipitation, mechanical filters, separately or in combination, and with or without the addition of germicidal lamps in the system.

1. Air washing

Air-washing systems are elaborate, space consuming and usually quite expensive, although reported results are good.

2. Electrostatic precipitators

Electrostatic precipitators or electronic-filtration is quite popular and offers both advantages and disadvantages. The initial installation is substantial.

Unless an automatic system is installed to wash the precipitated dust from the precipitators, frequent maintenance is necessary. The precipitated dust particles must be washed free and the electrical system kept clean. Electrostatic precipitators remove particles of fractional micron size and are an effective filtration system. Short-circuiting, although an infrequent occurrence, could render the system inoperative. Temporary power failure also would stop the filter system from functioning. Therefore, unless one is prepared to face some of these possibilities and eventualities, the safest and possibly most reliable, the mechanical filter, should be utilized.

3. Mechanical filtration

There are innumerable filter materials, and manufacturers of reputable products, and it is difficult to ascertain which filter is best. In general, some of the better filters are composed of Fiberglas and are designed to be as effective and, in some instances, more effective than electronic or electrostatic types. They filter out fractional micron-sized particles and offer the distinct advantage of being trouble-free, since there is nothing to go wrong mechanically. Pre-filters, which are standard on most air systems, are recommended,

since these will remove the larger sized particles and thus prolong the life of secondary bacterial or fractional micron particle filters. The smaller the particle removed, the more resistance offered by the filter, so that a satisfactory medium is sought to prevent unnecessarily oversized motors and blowers to force the air through the filter. Empirically, 0·7-0·9μ seems to be the common specification for particle removal. Controlled data are needed to verify this as well as many other common practices. Actually, the question has arisen concerning the need for fine filtration and it seems to be most justified where conventional colonies are in proximity. It adds an additional margin of safety further to isolate air systems, even though only clean colonies may be present, and it appears to be a good precaution to take.

B. VENTILATION

Ventilation is another controversial issue which has been regulated by empiricism. Animal laboratories have complete air changes ranging over periods from every 2 or 3 min to every 12 or 15 min. They utilize from 10% to 100% fresh air at all times with proportionate recirculation. What is ideal? What is adequate? What is minimal? Nobody seems to know for sure. There are, however, accepted practices that have proved reliable and workable through the years.

1. Separate air systems for each area

The optimum is, of course, a separate air system for each animal area supplying 100% fresh air at all times. This technique provides maximum ventilation and odour control and adds the safeguard that in the event of mechanical difficulties only one area rather than all is effected. It is the most costly to install but it provides a private air supply to each area without the possibility of cross-recirculation between animal rooms.

2. Single air systems

Another approach is a single system which services multiple areas on a 100% fresh air basis with no recirculation. This necessitates a common air supply duct to all areas which, in emergency situations, may allow backdraughts to occur from area to area. Recirculation of air in latitudes where temperatures reach extremes in winter and summer is a major economic consideration. Since the room temperature remains constant regardless of outside temperatures, the cost of heating zero-degree air or cooling 90°F air to a temperature of 75°F and exhausting it without re-use is extremely costly and somewhat wasteful. The word recirculation has attached to it an undesirable connotation but actually if the recirculated air passes once again through both primary and secondary filter banks it should theoretically be as pure as the original air.

3. Recirculation

If the filters are initially relied on to bring air in from the atmosphere, recirculation of air within a given area appears reasonable. Studies in germ-free, or gnotobiotic, life have shown us that isolators containing germ-free animals can function in a contaminated atmosphere, since the filters remove all micro-organisms. Thus, recirculation of air through good fractional micron filters in SPF colonies should be free of the danger of contamination. The ventilation system can be designed to perform its primary function as well as the additional functions of cooling, heating and humidification. This appears most desirable and has many efficiency advantages over a separate system for each facet of environmental control. Most of the recently designed and engineered systems are total year-round units and accomplish the complete conditioning of the environment through a common duct and air-handling system.

C. DUCTWORK

Ducts are usually fabricated from galvanized steel although more costly aluminium ductwork is also used. Aesthetics would suggest concealed ducts, but concrete structures have no air space to conceal them and they are therefore surface mounted. With some architectural ingenuity, ducts can be located so as not to be objectionable to the eye when surface mounted. Nevertheless, exposed ducts are readily accessible for initial decontamination purposes as well as in the event of restocking resulting from an unfortunate contamination.

D. COOLING

Cooling is usually accomplished by refrigeration systems. The most efficient is the direct cooling of air by passing it through refrigeration coils. Cooling can also be accomplished *via* a chilled water system and can work fairly well when the temperature and humidity extremes are not great and chilled water can be made available. When year-round availability is sought in a cooling system, evaporative condensers for cooling the refrigerant are installed inside, out of the weather. When weather conditions in the area are temperate, an outside water tower can be used and water pumped down during the cold season. However, this will prohibit the use of the cooling system on the occasional unseasonably warm day in spring and fall.

E. HEATING

Heating is most efficiently accomplished in a combined heating and cooling system by the use of steam coils in the air unit. These coils are in series with the cooling coils so that heating or cooling can readily be provided for. Hot water can also be used in the coils, but since steam is usually available and can be controlled more finely with modulating steam valves, it is probably the best method to choose.

F. HUMIDIFICATION

Humidification is best incorporated into the total system. Cold or hot water spray nozzles can finely atomize water into the air stream. For maximum efficiency, steam provides the best source of water for humidification. A simple system liberating steam by means of a motorized steam valve or a steam solenoid activated by a humidity sensing device in the animal area regulates the relative humidity. There are many efficient devices commercially available for area humidification independent of a central system. These units operate on cold tap water, automatically replenished by a float arrangement. Water is picked up from a reservoir and broken up into small particles by a high-speed impeller rotating at over 3,000 rev/min. These units work well and are recommended where centralized humidification is impossible.

G. DEHUMIDIFICATION

Dehumidification is costly but necessary in locations where high temperatures and humidities prevail seasonally. It is usually accomplished by cooling the air to condense out the moisture and then reheating to room temperature. The double expense of cooling and heating means that dehumidification is sought only when conditions are extreme.

H. CONTROLS

Controls, needed to operate the factors contributing to the total environment, are quite simple.

1. Five sensing controls in each area

Five individual sensing controls in each area seem to suffice, namely: heating and cooling in one, humidification, dehumidification, low-temperature and high-temperature alarm. The heating-cooling instrument is set to maintain 74°-78°F (23·3°-25·5°C). It calls for heat below 74°F and for cooling above 78°F. The humidification control is set to maintain humidity above 50% relative humidity, while the dehumidification control need not be set to operate until 65-70% relative humidity is reached. At this level, technicians find conditions uncomfortable and animal bedding becomes damp.

The low-temperature alarm thermostat can be set at 70°F (21·1°C) while the high-temperature alarm instrument may be set at 80°F (26·6°C). These are safe limits, since any slight deviation from them is still without danger.

It is recommended that all thermostats and sensing devices in the animal areas be proof against tampering so that they cannot be changed by unqualified personnel.

2. Alarms and emergency power

In the event of power failure, alarms should summon a watchman to start emergency generators; also, in power failure, cessation of the air system

should activate spring closure dampers on all ducts directly leading outside to prevent natural backdraughts.

If the budget permits its installation, an automatic power transfer switch can be activated by municipal power failures to start the emergency stand-by generator and transfer the power load. However, if watchman or maintenance personnel are on the premises, a manual switch-over is perfectly acceptable as long as the animal room temperatures do not become excessive and the essential supply of fresh air is not restricted unduly.

VIII. Entering Barrier

Personnel and supplies must enter the clean area through the barrier on a daily basis. Bedding, feed, air, water, shipping supplies, record-keeping supplies, laundry, personnel and their lunches constitute the majority of items that can conceivably bring in contamination if proper decontamination and entrance procedures are not worked out. Personnel entry requires considerable care and planning, since it is theoretically possible for contaminants to be on the person and possibly within the person. Again, since controlled data are lacking, we must assume that persons can carry pathogens and must be reckoned with in protection against possible contamination.

A. ENTRY OF PERSONNEL

Personnel entry probably varies more than the entry of any other item through the barrier. It ranges from simple changing of clothes in a single pre-entry lock to the use of a series of pressure locks followed by a prolonged shower.

1. General considerations

Shoes, more than any other part of the apparel, could come in direct contact with rodent pathogens and certainly with rodent faecal matter, which is almost universally found. Shoe removal, therefore, should be considered essential and shoes worn to work must never enter the barrier.

Outer clothing does not afford the same danger as shoes, but it is likely to come in contact with household pets, either in the home or in the family car, and therefore should also be prohibited from passing through the barrier.

From this point on, the risk is minimal but present. A fine line is approached when considering undergarments and indeed it is probably best to require complete divestment of clothing. This, however, presents a practical and psychological problem which should be given scrutiny. Unless thorough showering is to follow, the disadvantages outweigh the advantages. It is difficult, in some locales at least, to secure the services of female personnel who fulfil all the requisites for employment and who will agree to wash their hair at least once daily. Moreover, some female employees may be unwilling

to disrobe and shower in the presence of other female personnel. With men, community shower areas do not seem to present any problem.

If a group of reliable, interested people can be assembled who will agree to the shower, and there is assurance of an adequate replacement personnel with similar inclinations, a shower-room immediately preceeding the clean dressing-room provides good protection.

2. Winter weather difficulty

In northern climates, where weather conditions may be extreme, showering for either men or women may present a health problem. Arrival at work in sub-freezing temperatures followed by a shower could result in colds and absences from work. Of course, in more temperate climates this drawback is of less significance.

Since, in most instances, showering presents some type of drawback, the next best entrance procedure is as follows: three entry locks, or small rooms, are necessary as in the case of shower procedure.

a. First entry lock. All doors can be electrically interlocked so that the door behind must be closed before the next one is opened. In this way, during the peak entry and departure periods in particular, direct passage to the clean area by virtue of several doors being opened simultaneously is precluded.

The first lock should be under positive pressure and should have in it some type of aerosol sprayer. The aerosol should contain a contact insecticide and the sprayer should operate manually, or preferably automatically, when the outside door is opened. Good protection is thus afforded against insects which enter into the first chamber with personnel. Where boots and heavy coats are worn as added protection against winter weather, these articles are best left in the first lock.

b. Second entry lock. The second lock is under negative pressure so that air from this lock will not enter the clean lock, or third lock. In this second lock all clothing except undergarments are removed. In undergarments and paper slippers the personnel proceed to the next area leaving their slippers at the threshold.

c. Third entry lock. In this third and last lock entering personnel must first scrub thoroughly with hand brush and germicidal soap before touching anything else in the room. They then put on sterile clothing, including cap and shoes, before entering their respective work areas. Where both male and female personnel work within a single area, dual lock arrangements are provided.

3. Carrying lunches into clean area

Another consideration in the entry of caretaker personnel is the question of lunches. The two possibilities for consideration here are for technicians to

pass through the locks to eat outside the barrier, or for them to bring their food inside. Consistent with the gnotobiotic concept, that potential hazards exist each time the barrier is penetrated, eating within the clean area appears to be best. If eating outside the barrier was practised, the entry of personnel through the barrier would, in effect, be doubled each day, thus doubling the hazard. Since it is unlikely that food prepared at home, in the kitchen, contains rodent pathogens, it is a reasonable calculated risk to allow food to be carried from the home. Water-tight, rigid plastic containers can be supplied to each person for the purpose of transporting lunches to work. These containers may be passed in through a germicidal dunk tank or carried in with personnel who completely submerge the package just prior to scrubbing. In the latter instance, after clean clothes are put on, the lunch may accompany each person into the clean lunch area where it is left until consumed. Unopened packages of cigarettes are permitted to enter via the dunk tank. Since paper or wooden matches are almost impossible to sterilize, smokers are required to supply a cigarette lighter which is sterilized and remains inside.

4. Surgical masks

The question as to whether persons working with SPF animals should wear masks is one answered best by each individual production unit. Most producers of SPF animals are routinely doing without face masks. Some compromise and require them in situations of respiratory distress. At best they are of questionable worth and if worn should be used double and changed every $1\frac{1}{2}$ to 2 h; they are extremely uncomfortable to wear for prolonged periods, especially for people whose job keeps them active.

One of the major unanswered questions in the field of SPF production is what role, if any, is played by the human respiratory tract. Some speculate that rodent pathogens can be carried in the human naso-pharynx when personnel go from room to room. They theorize that humans are not a natural host for airborne rodent organisms but possibly may act as transitory resting places for such organisms. There are others who are very concerned with human pathogens and their possible ability to adapt to the rodent. It is apparent that speculation will continue until specific experiments are conducted in this field.

PPLO, pneumococci, streptococci, staphylococci, corynebacteria, viruses and the rest will continue to offer a challenge to both the animal breeder and the research investigator. It is the hope of many that research in this vital and perplexing field will be undertaken soon.

B. ENTRY OF BEDDING

Bedding is relatively easy to sterilize or decontaminate and can be handled in many different ways.

1. Varieties of bedding materials

There are actually, in the U.S.A. at least, many commercially available bedding materials packaged for immediate use. They are offered as highly absorbent materials and, in some cases, capable of neutralizing the ammoniacal urine odour. Some have, in the course of processing, been subjected to temperatures sufficient for sterilization and are then packaged in plastic or waterproof enclosures to prevent contamination.

The spectrum of bedding or litter materials varies from edible, organic, combustible substances to inert, inorganic, non-combustible agents. The selection of which bedding to use is governed by cage type, method of cage cleaning, disposal methods available, type of sterilization equipment on the premises, and the cost of the material which may be affected by geographic location.

In many rat-breeding, holding and research units, the animals are housed in cages with wire-mesh floors eliminating the need for bedding except in littering cages. Where disposal is accomplished by incineration, combustible bedding must be used. In areas where lumber is a major industry there are distinct economic advantages in the use of wood shavings and by-products. It is probably safe to say, for the United States and Canada at least, that wood shavings are utilized in greater quantity than any other single bedding material.

2. Methods of bedding sterilization

With a vacuum-equipped steam autoclave sterilization of wood shavings is a simple matter. This is true whether the shavings be packaged as loose bags or compressed bales. A vacuum of at least 26 in (66 cm) of mercury followed by steam at a pressure of 15 lb/in² (0·95 kg/cm²) assures complete penetration and sterilization. Utilizing the same sterilizing chamber, ethylene oxide gas can be used as the sterilizing agent. This form of sterilization has become fairly popular in the U.S.A. during the past five to ten years. Its primary advantage is in the sterilization of thermolabile products, since with 20% ethylene oxide the cycle can be accomplished at as little as 140°F (60°C). Its main advantage in non-thermolabile products is that it is a relatively dry cycle and does not dampen the load. High-pressure steam is not required, since the sterilization process is effective at low temperatures and pressures. There have been reports that this particular mixture has not proved entirely satisfactory in the U.S.A. In Europe a combination of 90% ethylene oxide in conjunction with a temperature of 70°F (21·1°C) has proved quite effective and appears not to have toxic effects in wood shavings. It must be noted, however, that this mixture is very inflammable, unlike the 20% mixture, and great care must be taken to prevent fire and explosion. The main drawbacks when using ethylene oxide for bedding, particularly shavings, are the cost, the length of time required and the retention of the gas. At low temperatures of 140-160°F

(60-70·1°C), exposure time of the bedding to ethylene oxide must be at least 4 h. After the cycle is complete and a final vacuum drawn to remove the gas, it is not uncommon to find residues of ethylene oxide that can cause irritation to small rodents. In the case of a closed isolator system, residual ethylene oxide in the bedding can be toxic and fatal to valuable germ-free animals unless properly aerated prior to use.

Whether steam or gas is used, the vital step in the sterilization process is the vacuum stage which prepares the load for thorough penetration. It is almost impossible to penetrate a bale of bedding to any depth without first drawing a vacuum. It would be necessary to spread the bedding thinly and loosely on trays for steam sterilization without the vacuum cycle. This is time-consuming, costly and somewhat outdated.

C. FEED

The preparation, pasteurization and handling of feed is not as simple as that of bedding or other materials. The major difference is the possible damage of vital nutrients which ultimately affects growth and reproduction. There are many systems that have been tried in the search for a non-nutrient damaging process and it may be well to consider them separately for the purpose of evaluation.

1. Ultrasonics

Ultrasonics have been attempted on a limited scale and offer some encouragement. Information is lacking as to current status, but previous investigations were encouraging.

2. Ionizing

Radiation has been proved to be an excellent method for sterilizing foodstuffs. It effects sterilization without trace of nutrient damage and therefore is ideal. Its drawback, currently, is its cost of operation. The equipment required is extremely costly and involves a technical area where specialized training is necessary. There is little question that some time in the future further research will make available equipment and technology which will enable animal diets to be sterilized by radiation.

3. Microwave heating

Utilizing high-frequency radio energy is a very satisfactory method of pasteurizing diets without nutrient damage. Tests by the author have proved that vegetative forms of bacteria can be effectively killed at 210°F (98·8°C) with microwave heating without damage or significant reduction of the most thermolabile nutrient, thiamin. Growth and reproduction studies for three generations failed to detect any nutritional imbalance that may have been missed with chemicals and bio-assay. Present available equipment permits

limited processing of under 200 lb (90 kg) per h. The cost of operating the equipment, service replacement of parts and amortization of the principal constitute but a small fraction of the total cost. The major drawback for large consumers of feed is its slow productive capacity. Also, being a highly special ized electronic device, it often requires service by specialists. When conveyor equipment is designed and perfected to be competitive with large multi-purpose autoclaves, and the capacity per hour is increased, microwave heating will offer a safe method for pasteurizing animal diets.

4. Ethylene oxide

Ethylene oxide, a proven bactericidal and parasiticidal gas, will penetrate and sterilize animal feeds most effectively. However, its use is prohibited by the destructive characteristics of the gas to essential nutrients such as methionine and histidine (Hawks and Michelson, 1955). There is additional destruction of B vitamins, but this is not as serious since the cost of prefortification with B vitamins is negligible. When dealing with the amino acids we are unfortunately involved in a rather expensive and wasteful situation. Prefortifying with costly ingredients, only to lose a large percentage of them in the sterilization cycle, is economically unsound. Ethylene oxide further has the undesirable characteristic of continuing to destroy nutrients while the food is being stored after the sterilization process. Gas sterilization may yet prove practical when another agent is found that is non-toxic, bactericidal and not damaging to nutrients.

5. Dry heat

Heat, in one form or another, has been studied the most and provides a reliable form of feed pasteurization or sterilization. The effects of heat on nutrients has been well described not only as it relates to animal diets but most commonly where human nutrition is involved. It is, therefore, a fairly simple matter to pasteurize animal diets with either dry-heat baking ovens or by steam. The method utilized will depend upon the quantity of feed required and the type of heat source available. There are manufacturers of animal diets who utilize large rotary baking ovens to cook their diets. Their formulae contain a lot of water and are usually prefortified with thermolabile vitamins so that after baking there are still generous amounts of nutrients present. These manufacturers then package the feed in a clean plastic bag before using a multi-walled paper outer wrapper. This gives reasonable assurance that the baked food reaches the user in as clean a condition as when it was manufactured and has not been contaminated *en route*. As long as both outer bags and the sealed inner plastic bag remain intact the user can be assured of its safety. Upon receipt of the feed the outer bag is stripped off and the plastic bag containing the baked food is passed through a germicidal dunk bath or a low-pressure steam chamber into the clean area. This is a proven process,

since bacterological examination has failed to uncover anything other than non-pathogenic spore-formers which are of no practical significance. There is, of course, the possibility that contamination could occur some time between the actual baking of the diet and its entrance through the barrier into the clean area. Consequently, pasteurization immediately preceding entrance through the barrier and use approaches the optimum situation.

6. Steam

This can be accomplished by baking the diet with steam in a two-door autoclave loaded from an outside receiving area. After the pasteurization cycle is completed the door on the clean side is opened and the food unloaded without the risk of outside contamination.

There are several factors to be considered before attempting such a process. First, there is some limited nutrient destruction which has to be compensated for in the initial manufacture of the diet. Second, if a pellet is used, penetration can only be accomplished by drawing a high vacuum on the chamber prior to the introduction of steam. Third, a rather delicate balance between vacuum, steam temperature and time is somewhat critical to control pasteurization, nutrient destruction and caramelization of the diet. There is work currently being performed at the author's laboratories which may be published in the near future to describe more accurately the techniques involved.

In the field of gnotobiotics feed processing presents special problems since complete sterilization is necessary, thus placing considerably more stress on the nutrients in the diet. Work is continually being performed to construct the ideal formula capable of withstanding temperatures in excess of 250°F (121·1°C). The limited production of germ-free animals is partly attributable to inadequate nutrition which may possibly be coupled with the lack of micro-organisms in the gastro-intestinal tract. Often, when a contamination occurs in a germ-free system, reproduction increases even in the case of mono-contamination.

D. OTHER MATERIALS

All other materials which enter the barrier system should be decontaminated by heat, gas or a liquid germicide. The choice will depend on the nature of the product and the quantity required in the daily operation of the colony.

IX. Leaving Barrier

Precautions must be taken when materials are removed from the building.

A. POSITIVE-PRESSURE CHUTES

In the case of animals or small materials, safety exits or hatches must be designed to prevent the entry of vermin and unfiltered air. This can readily be accomplished by positive-pressure chutes which deliver a swift flow of air from inside the barrier whenever the outer hatch door is opened. To eliminate

human error the air flow can be automatically activated, and as long as the outer hatch door is open, the air continues to flow.

1. Animals in shipping containers

Shipping boxes, screen wire and wire for box stitching are sterilized by ethylene oxide. Packaging animals for distribution, if accomplished in the clean area, offers the flexibility of last-minute changes in a request for animals. In situations where packaging is performed outside the barrier the sterilization of the boxes is, of course, unnecessary. It does mean, however, that animals must leave the barrier for the packing process and can never return, even in the event of cancellation of animal requests, or last-minute reductions in the number of animals required. Individual situations will dictate which procedure is best.

B. EXCREMENT AND SOILED BEDDING

Since anything entering or leaving the barrier presents a hazard, it is well to consider means other than hatches when dealing with large or continuous quantities of materials such as soiled bedding.

In the case of excrement and soiled bedding, in a large production colony, removal is a daily and almost hourly procedure. Automation in this area is not only labour saving but, if engineered well, minimizes and almost eliminates the risk of contamination entering. Several systems are currently being employed in SPF production units that automatically and safely remove excrement from within the barrier and deposit it outside.

1. Auger conveyor

One situation utilizes an auger-type conveyor built into a concrete pit beneath the floor. The pit is covered with tight-fitting steel doors which are opened only to fill the V-shaped excavation with material. When the steel doors are closed the screw-type conveyor carries the soil outdoors through a tightly fitted opening in the outside wall. A manure spreader is located so as to receive all material from the pit, thus facilitating further handling and distribution. The main drawback is that everything must be carried or transported to the one pit and consequently additional handling is required.

2. Water-flushing system

Another automated system catches the droppings on a long battery of pans pitched toward the centre. A water-flushing system washes all the droppings to the centre of the pitched dropping trays into a central drainage system and on into the sewerage pipes. This, of course, necessitates the use of wire-mesh floors in cages rather than solid bottoms with bedding. It can be operated manually, semi-automatically, or completely automatically, depending on the individual requirements and budgetary limitations. Probably the single most important prerequisite for such a system is a sewerage system of

sufficient capacity to deal with the large volumes of material generated by any sizeable production unit. If such sewerage capacities are available, the labour saved probably amortizes the initial cost of equipment. Odour levels are minimized, at negligible cost, because of the ability to clean at frequent intervals.

3. Vacuum system

The following method of excrement removal is a result of the author's 10 years' experience in the use of pneumatic cage cleaning.

Since cage cleaning, especially involving wood shavings or wood by-products, creates a generalized dust problem, the initial step is to develop an automatic system which can remove soiled bedding and excrement with the

FIG. 1. Use of vacuum hose in removing soiled bedding from rat cages.

creation of minimal amounts of dust in the area. Also, it is desirable for such a cleaning system to remove the bedding directly from the cage and deposit it outside the building, thus eliminating the necessity for additional openings from the building with its coincidental hazards of vermin entry. After considerable trial, error and experimentation, a pneumatic system comprised of a network of sealed, air-tight steel tubes is the end-result. Commercial vacuum cleaning companies stock all the necessary components, since commercial vacuum installations in hotels and industrial buildings are fairly routine. Bedding offers its own special problems since it is moist when soiled and can, through faulty use and design of equipment, clog the lines. Also, it has a tendency to bridge in the centrifugal hopper-like separators which are an integral part of a pneumatic system of this type. Through testing, properly sized separators and hopper sizes have been developed, and through careful management and control of the system's use, a most efficient, complete cleaning and disposal system has been developed. This system is of particular benefit in SPF colonies where the individual animal rooms operate as separate independent units. Cage cleaning can be undertaken anywhere within a series of connected buildings and, *via* many hundreds of feet of pipes, deposit the excrement as far away from the area cleaned as desired. An adjunct to this system can be an additional blower or mechanical conveying device which transports the waste to a continuously operated incinerator.

Through the use of specially designed nozzles and cleaning tubes, patterned after household and industrial cleaning tools, general room housekeeping by way of floor, ceiling and wall cleaning can be accomplished.

X. Summary

The need for clean animals, free of their commonly occurring pathogens and parasites, has long been established. Current information indicates that the caesarean delivery concept is the most efficacious approach, since test and eradication as well as chemotherapy achieves only partial removal of the common animal diseases.

Dedicated workers in germ-free research pioneered the current concepts and techniques used by producers of SPF animals. They discovered that the foetal placenta was a highly efficient filter of micro-organisms and that by utilizing aseptic caesarean techniques all detectable animal infections and infestations could be eliminated. It was also proved by their technology that most diseases and parasitic infestations must therefore be transmitted from mother to offspring at birth or shortly thereafter.

Henthorne, Nelson, Innes and others have demonstrated on a small scale that once freed of common diseases, colonies could be kept clean, utilizing rigorous quarantine procedures. These included the safe entry and exit of the supplies and tools necessary for day-to-day colony operation.

Commercial producers expanded upon the basic concepts developed,

in an effort to make available large numbers of defined animals for biological research, thereby reducing variations in experimental results. Conventional colonies still greatly outnumber SPF or clean colonies, but the gap is closing steadily. It is but a few years ago that caesarean derived, clean animal colonies were a research novelty. They were termed abnormal by many. Knowledgeable people stated that findings resulting from their use could not be extrapolated to humans. Believers in this theory felt that laboratory animals have a normal incidence of overt and latent infections and we must learn to live with them.

A shift in this belief is now being demonstrated since it has been proved that common infections and parasites can be removed entirely from animal populations, utilizing gnotobiotic techniques. These animals can be maintained in their healthy state by rigid quarantine and environmental control.

Current feeling is that much of the experimental work done in the past is invalid due to the inability to distinguish between experimental effects and abnormalities caused by the high rate of natural morbidity found in most conventional colonies. Research investigators are no longer hesitant about using caesarean originated colonies. On the contrary, clean animals are being specified in ever-increasing numbers with inadequate supply being the only limiting factor. When proper maintenance facilities can be provided at the user level, research investigators will insist upon clean animals and in many instances postpone research projects when the supply is short rather than revert to the use of conventional animals.

Opinion is on the increase, as each month and year passes, that the clean SPF animal is truly more representative of a normal animal than its predecessor, the conventional animal. Thus, the expression normal animal eventually will apply in time only to the clean animal.

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Chapter 5

Germ-free Animals

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I. General Introduction

The application of germ-free animals to problems in the nutritional, physiological, bacteriological and serological fields has increased in the past few years. Germ-free animals which are free from microbial contamination offer experimental approaches which cannot be obtained by other means, and this consideration justifies the expense and exacting procedure necessary to rear them. Many excellent reviews and monographs on germ-free animals have appeared which will be referred to in the text. It is due to the pioneering work of Reyniers and his colleagues—Gustafsson, Miyakawa and Glimstedt —that the stage in germ-free work has now been reached where most of the difficulties in rearing have been overcome so that they are now no longer laboratory curiosities but valuable research tools. With the introduction of plastics, the price of the apparatus has been drastically reduced and according to Trexler (1961) several animal breeders are rearing germ-free animals

with the prospect that they may be commercially available in the near future.

Reyniers *et al.* (1949a, b) have proposed a new terminology for germ-free work and use the word "gnotobiote" (known life) to cover not only the germ-free state but also the germ-free animal living in association with certain known bacteria or other foreign organisms. Since it is the purpose here to discuss methods of obtaining and rearing germ-free animals, and not their experimental applications, the term "germ-free" is used throughout as described in section four (p. 165). The application of germ-free animals to research problems has been ably discussed by Phillips and Smith (1959) and by Mickelsen (1962).

II. Apparatus

A. Introduction

The purpose of all germ-free rearing apparatus is to create and maintain a barrier between the conventional surroundings and the experimental animals. It is obvious that to some extent the functional efficiency of the apparatus will depend on the environment, and a bacteriologically clean laboratory is desirable for the housing of the germ-free rearing units. Thus, the housing laboratory should be a room to which clean filtered and dehumidified air is supplied at a slight positive pressure, and facilities such as steam and electrical points are present. The walls should be designed so as not to collect dust and should be easily washed. There should also be an adjoining room for cleaning the apparatus. This is necessary, as experiments in which defined floras are implanted in animals could flood the atmosphere with organisms on dismantling the apparatus. Workshop facilities are also desirable since emergency repairs may arise and the construction of special devices is often necessary. Humidity control is a problem in rearing animals in the confined environment of a germ-free unit or isolator. Diet, faeces, urine and the respiration of the animals tend to elevate humidity. Control can be effected by the supply of dehumidified air and removal of waste products. Once the atmosphere in the unit becomes saturated it takes some time before it can be brought down to a normal level, even if the air flow is increased.

The apparatus to be described is modern apparatus which is currently in use and has been adequately tested. Although the concept of a germ-free rearing unit is simple—an hermetic compartment capable of housing animals and equipped with a mode of supplying sterile food, water, etc.—in order to obtain a workable design attention to technique is essential. The apparatus must be gas-tight; filters which sterilize air, and air outlet filters must not be allowed to become wet. Steam under pressure is the method of choice for sterilizing units and this consideration has influenced the design. Two types are in use: the autoclave type and those which can be autoclaved. Also discussed are plastic units which are sterilized by chemical means.

B. AUTOCLAVE TYPE—REYNIERS UNIT

This apparatus (Reyniers, 1959) has been used extensively at the Lobund Institute and elsewhere and was the first apparatus used on a large scale in successful germ-free rearing experiments. The isolator consists of a cylindrical stainless steel tank, 35 in (89 cm) in diameter, fitted with two round windows and two sets of neoprene gloves which are off-set so that two workers can cover the whole interior. Air is sterilized by filtration through glass wool (F.G. 50 material, American Airfilter Co., Louisville, Ky.). Entry and exit of materials from the unit is effected by means of a small jacketed autoclave (food-clave) fitted with doors at each end. This autoclave can also serve as a link between two isolators. In addition some of the isolators can be joined end to end. Various refinements have been made with series II types; the viewing position is larger, 12 in (30 cm) diameter, and set at an angle for easy viewing, and an external system for supplying sterile water is available. This latter feature is a great boon in the operation of the apparatus because it is standard practice to load it with all materials capable of withstanding heat sterilization before sterilizing the unit, and water takes up a large proportion of space.

Prior to operation the apparatus is loaded and tested for leaks. An advantage of a high-pressure isolator is that leaks may be determined very efficiently by testing under high pressure (25 lb/in^2; 1·94 kg/cm^2) except in the gloves. One of the most sensitive methods consists in the use of a freon detector. Any leaks round gaskets are stopped by tightening the joints or changing the gasket. Leaks from pores in the stainless steel must be located exactly by use of soap solution and soldered. Once the apparatus is gas-tight, sterilization can proceed. The food-clave is sterilized with the main unit by opening the connecting door. If the germicidal trap is to be used, this is also sterilized with the main unit. The germicidal trap arrangement is a simple downward inclined tube fitted with a cap. After sterilization the cap is removed after the tube has been immersed in a container of germicide. Insulating blankets can be placed over the apparatus to reduce heat loss during sterilization. Caps are placed over the glove-ports during sterilization.

Reyniers (1959) has recommended the following sterilization cycle. A vacuum of 20 in (508 mm) of mercury is held for 10 min. Steam is then allowed to flow through the filters into the apparatus and into the jacket of the subsidiary autoclave until a temperature of 122°C (252°F) is obtained and held for 30 min. Temperatures of autoclaving are monitored by placing thermocouples in the main chamber and elsewhere, and recorded. During both vacuum and pressure phases, care must be taken to maintain an equal pressure on both sides of the gloves. This is effected by applying compressed air or vacuum to the gloves *via* a valve in the glove-port caps and observing the gloves through the window.

The pressure is slowly dropped to zero and a vacuum of −20 in Hg applied

for 10-15 min. This is sufficient to dry out the isolator and filters. The filters have a built-in electrical heating system which is used to dry them completely. Air is then passed through the filter and the apparatus is ready for use.

Several models have been described, the most important being the surgical isolator. This is an isolator supplied with two sets of gloves and two windows. A horizontal partition divides the interior into two halves. The partition has a circular hole of 14 in (36 cm) diameter in line with a platform in the lower section which can be raised or lowered. A door leads into each section. After sterilization, a layer of cellophane, nylon, or mylar is placed over the dividing hole and maintained in place with a rubber band. The prepared animal is introduced through the door at the lower end of the apparatus and placed in position on the platform. The platform is raised until the abdomen of the animal comes in contact with the plastic. A sterile surgical adhesive can be used to stick the skin of the animal to the plastic.

The apparatus is fitted with an electric cautery with which an incision is made through the plastic and skin of the animal. This procedure does not expose any contaminated surface to the sterile interior. The uterus is removed whole, placed on towels, and the young removed. The umbilical cords are sealed with the cautery and the animals handed to the assistant wearing the other pair of gloves, who cleans and revives them. They are passed into a previously sterilized and attached rearing isolator which is then disconnected from the operating unit.

A modification of the above procedure has been described (Parks, 1958b) enabling successive animals to be operated on without resterilizing the isolator. A stainless steel ring was placed over the operating area. After completion of one caesarean section, a second sheet of plastic was installed over the first and held in position with the ring; the animal was removed and the apparatus was ready to receive another. As many as four successive operations were performed without the necessity of resterilizing the apparatus. Contaminations were extremely rare.

The Reyniers unit is manufactured commercially by Renyiers & Son, Chicago, Illinois, and priced at $6,100 for the rearing unit and $6,400 for the surgical unit. Germicidal traps, sterilization control panel and recording cart are extra.

In summary, the Reyniers unit is a dependable and extremely sturdy apparatus. Most of the results obtained in past germ-free research have been made using this apparatus. However, the disadvantages are the very high initial cost and the bulkiness of the apparatus. Once installed, this apparatus should give excellent results.

C. AUTOCLAVABLE TYPE

1. Gustafsson unit

This apparatus differs from the Reyniers apparatus in several respects. It is a rectangular shaped tank made of light-gauge (2 mm) stainless steel, fitted

with a plate-glass top. This ensures good viewing of the interior. Air is sterilized by incineration at 300°C (572°F) by passing through a heated carborundum column. The sterilized air is cooled by passage through a pipe running external to the apparatus and air from the apparatus is returned to the sterilizer, where it is reheated before entry to the atmosphere. Entries to and from the apparatus are made *via* a germicidal trap. Gustafsson (1959) has also used a subsidiary autoclave which leads into the germicidal trap on some apparatus.

Two types of germ-free rearing isolators have been used. The smaller type has a volume of 200 l and holds ten rats. The larger type has an internal capacity of 700 l and is capable of housing twenty to thirty rats.

As with all rigid apparatus, the stainless steel has to be carefully welded and all corners ground to a radius to minimize faults in the welding. The gloves are made in two parts; the sleeve is made of heavy-gauge rubber which is bolted into the frame by a stainless steel ring. The gloves are lighter gauge rubber and neoprene and are clamped to the sleeves by means of a cuff ring. The joints may be sealed with rubber solution.

a. Operation. The apparatus is sterilized by autoclaving in entirety (Gustafsson, 1948). A vacuum autoclave is adapted so that germicide contained in a tank above the autoclave can be run into the germicidal trap of the apparatus, without opening the door of the autoclave, by means of a valve passing through the door of the autoclave. An electrical connection passes through the autoclave to the incinerator of the apparatus. The apparatus is assembled through the top with cages, water and other materials which will withstand autoclaving. The glass top is placed on a rubber gasket and secured in place by screws. The apparatus is then placed in the autoclave with no germicide in the trap. The electrical connection to the air sterilizer is made and turned on; the pipe from the tank of germicide above the autoclave is connected to the germicidal trap. The autoclave is then evacuated to −20 mm Hg. This also evacuates the apparatus. Steam is then entered until the temperature is 121°C (249·8°F) which is held for 30 min. The pressure is allowed to decrease gradually and the germicide is heated to 80°C (176°F) by steam from the autoclave. When the steam pressure in the autoclave is near zero, the germicide runs into the trap of the apparatus. This disconnects the interior of the apparatus, which remains sterile, from the autoclave. Air is then blown through the air sterilizer for 12 h to dry and cool the apparatus. The whole sterilization and drying procedure takes about 24 h. Germicide is also run into the groove at the top of the apparatus and surrounds the gasket between the glass panel and tank.

b. Mode of entry. The small apparatus is fitted with a germicidal trap. This is filled with 0·1% cetyl pyridinium chloride solution. Other quaternary ammonium compounds and other germicides will serve, but a frothy substance should not be used. The germicidal trap is designed to be a barrier only and

not to deal with any appreciable bacterial load. All materials should be surface-sterile before entry. Ampoules containing vitamin solutions or bacterial cultures must be degreased and sterilized by immersion for 2h in chromic acid solution, and other entries such as containers of diet should be wrapped in paper or nylon before sterilization to keep the surface sterile. The germicide should be assayed on occasion and care should be taken to avoid contaminating the germicide while removing waste from the apparatus. With due care, the germicidal trap should not be a cause of contamination.

Gustafsson (1948) has given details of an operating procedure for obtaining first generation germ-free rats. This differs from the Reyniers system in that the skin of the abdomen of the mother animal was excised and stretched over a ring and attached by hooks, leaving the body-wall bare. The animal was then placed at the base of a small operating apparatus to which the ring was bolted, so that the subcutaneous tissue covering the ring was pressed against the edge of the operating apparatus forming an hermetic seal. A shallow space exists between the abdomen of the animal and a plate leading into the operating unit. This space was flushed with iodine-alcohol solution for 10 min, the plate removed and a hysterectomy performed, during which the placenta was squeezed to stimulate respiration. The young were transferred to a rearing unit in a stoppered glass tube. Gustafsson (1948) estimated the operating time as 50 min and reported good results with this technique.

There do not seem to be any special manufacturers of this apparatus, and workers who wish to acquire it must make their own arrangements for manufacture when they specify their own modifications. The price is approximately one-quarter that of the Reyniers unit.

In summary, the Gustafsson apparatus is an elegantly designed system which has given excellent results. There is no doubt that the germicidal trap can be a weak point leading to contamination unless used with care. Provision should be made in case of a burn-out of the air incinerator which may also result in contamination.

2. Autoclavable plastic unit

Lev (1961) has described a germ-free rearing unit made of nylon. This was designed for maximum simplicity. The apparatus (Fig. 1) consists of a tube of 0·002 in (0·05 mm) portex (Portland Plastics Ltd., Hythe, Kent) nylon 8 ft (2·4 m) long and 76 in (2·0 m) circumference, and 40 in (1 m) in length. Two incisions were made in the nylon tube to accommodate the neoprene gloves and no other sites of leakage at joints exist. One end of the tube is constricted round three 8-in (20-cm) stainless steel rings and dips into a tank filled with germicide. Air is sterilized by filtration through four layers of F.G. 50 glass-wool and enters and leaves the apparatus *via* pipes through the germicidal trap. The apparatus is loaded through the free end of the nylon tube which is secured by folding and wrapping tightly with rubber tubing.

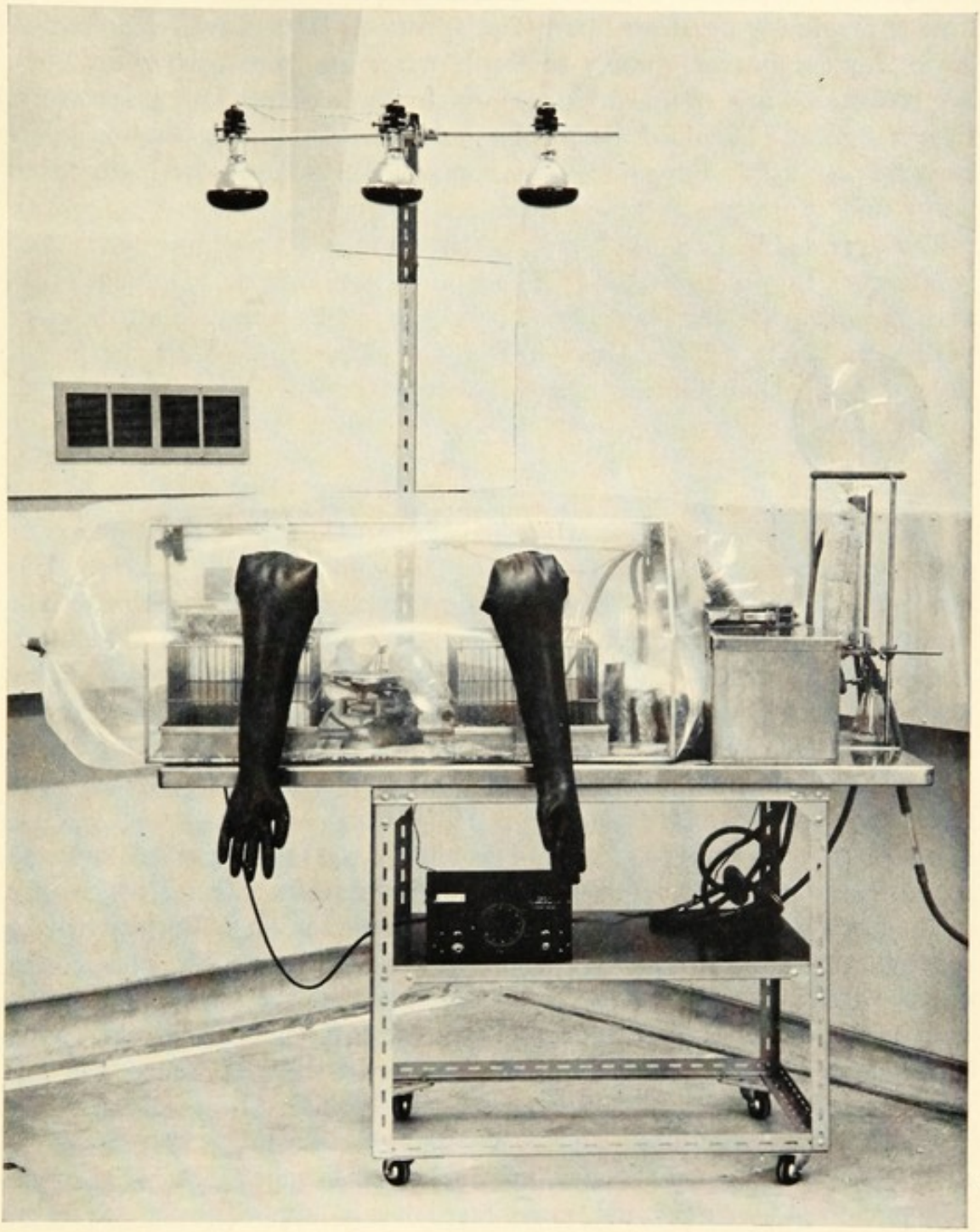

FIG. 1. Autoclavable Plastic Unit

The apparatus is sterilized in the same manner as the Gustafsson isolator (see above) except that no electrical connections are necessary. After autoclaving (17 lb/in² (1·2 kg/cm²), 30 min) and filling the trap with germicide, the air filter and apparatus are dried in the autoclave by air brought through from a previously dried air filter. The apparatus is then ready for use. A device for the internal supply of sterile water has been used (Lev 1963). Water is stored in a 10 l flask (Fig. 1) which can be changed when necessary. Contamination is avoided during this procedure by autoclaving the joints between the flask and apparatus. Space inside the apparatus normally taken up by cans of water is therefore freed.

The apparatus has worked well in the rearing of germ free-chicks and guinea-pigs. In addition to the method of sterilization, the advantage of an autoclavable nylon unit is that it is inexpensive and has the good attributes of flexible units (see below). The nylon tube, available as 38 in (96 cm) layflat tubing, is transparent, tough and easily replaceable. It is cheap enough (approximately 30 shillings) to replace after each experiment.

D. CHEMICALLY STERILIZED PLASTIC UNITS

Plastics are cheap materials which have been used for the construction of germ-free rearing apparatus (Trexler and Reynolds, 1957). Trexler (1959) has listed the advantages of the use of flexible plastics over rigid materials. Plastics are lighter than steel and flexibility is of considerable aid during manipulations in the isolators. An important feature is that because of the flexibility it is virtually impossible to obtain a negative pressure in the apparatus which can occur when removing the gloves after working in the isolator. The constant positive pressure would help maintain sterility for some time should a small leak occur.

Apart from the low cost, all-round visibility, etc., plastics can be fabricated to any shape for a particular purpose, in contrast to steel apparatus, which once made is relatively permanent. Thus large animals can be accommodated by scaling up small apparatus for a negligible increase in cost. The plastic is of course vulnerable to puncture. However, in practice, the gloves are the most vulnerable portion of the equipment and there is little danger of puncturing the plastic. The apparatus described by Trexler and Reynolds (1957) is manufactured commercially by the American Sterilizer Co., Erie, Pa.

The American Sterilizer isolator consists of a chamber $28 \times 28 \times 40$ in ($71 \times 71 \times 102$ cm) made of heavy polyvinyl chloride which is sealed gas-tight and supported by an external frame. Sterile air is supplied to the apparatus through a glass-wool filter and escapes through a liquid trap. Fig. 2 shows a semi-diagrammatic representation of the apparatus. Entries are made through a 12-in (30-cm) diameter door at one end, and this extends to a tube which can be attached to a lock by means of pressure-sensitive tape. The tube is anchored to the base of the unit and the lock is used

for entering or removing material. Polyvinyl plastic is heat labile and sterilization is effected with a 2% aqueous solution of peracetic acid plus 0·1% sodium alkylaryl-sulphonate. This mixture has been shown to inactivate spores within 30 sec in the liquid phase and within 10 min in the vapour phase (Trexler and Reynolds, 1957).

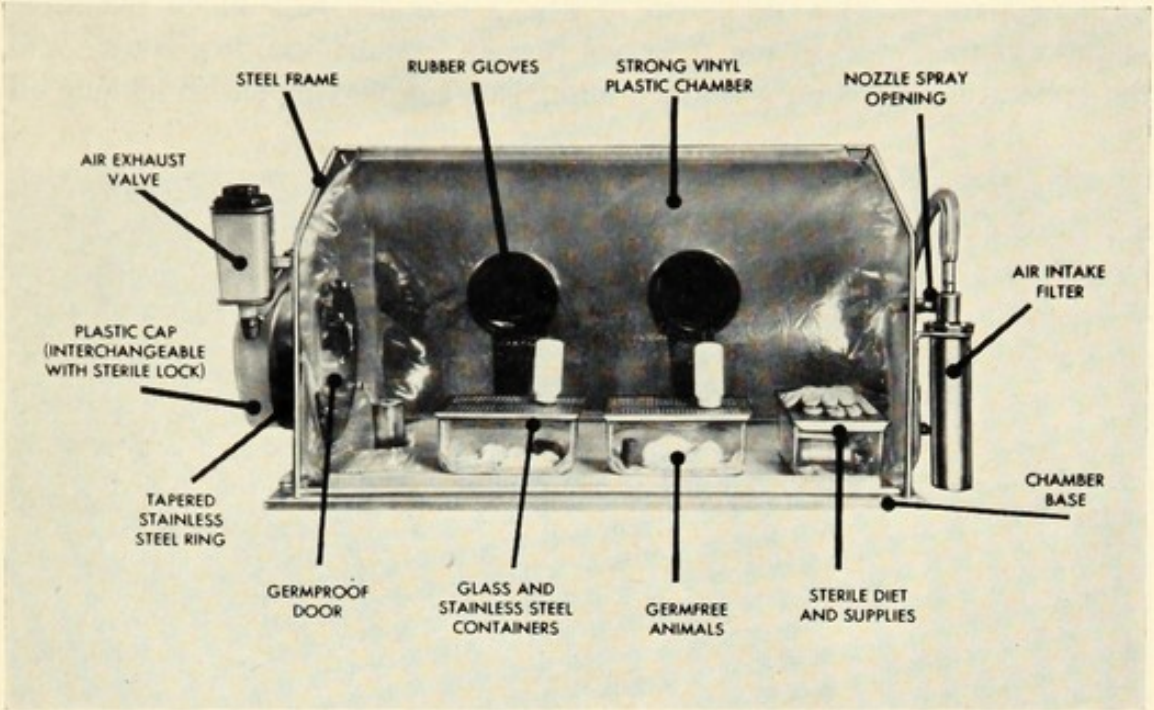

FIG. 2. American Sterilizer Co.—Plastic Germ-free Rearing Unit.

The sterilization of the unit is relatively simple. The air filter is sterilized separately by dry heat with the outlet end covered with mylar or other heat-resistant plastic and fitted into place. After washing with detergent, the interior of the chamber is sprayed with 2% peracetic acid until a dense mist is formed. This is left in contact with the apparatus for ½-1 h, after which the mylar separating the filter from the apparatus is punctured, and the peracetic acid is flushed out overnight with sterile air. Entries are made by placing surface-sterile material in the lock attached to the door-tube extension. A plastic cap is placed over the end of the lock and peracetic acid is sprayed into the lock through a small opening. After the ½-1 h contact time the germicide is flushed away and the material entered. A germicidal trap can also be fitted to the apparatus and an air incinerator is available and used, for example, to prevent the escape of pathogenic organisms into the laboratory.

A surgical unit is also available. This differs from the rearing apparatus in that two pairs of gloves are fitted and an operating area, covered by plastic, is placed in the floor. As with the rearing apparatus, tubes are sealed in the plastic which facilitates the provision of electrical appliances into the apparatus.

This system has been used successfully. The disadvantage in the mode of sterilization is that peracetic acid is an irritating and corrosive substance. The price is $895 for the apparatus as illustrated.

1. Trexler apparatus

Recently Trexler (1961) has described a simpler and cheaper version of the apparatus. This is shown in Fig. 3 and has very much the same features as the model in Fig. 2, except that no supporting frame is used and the apparatus stands on a wooden base. This can be adapted to include an

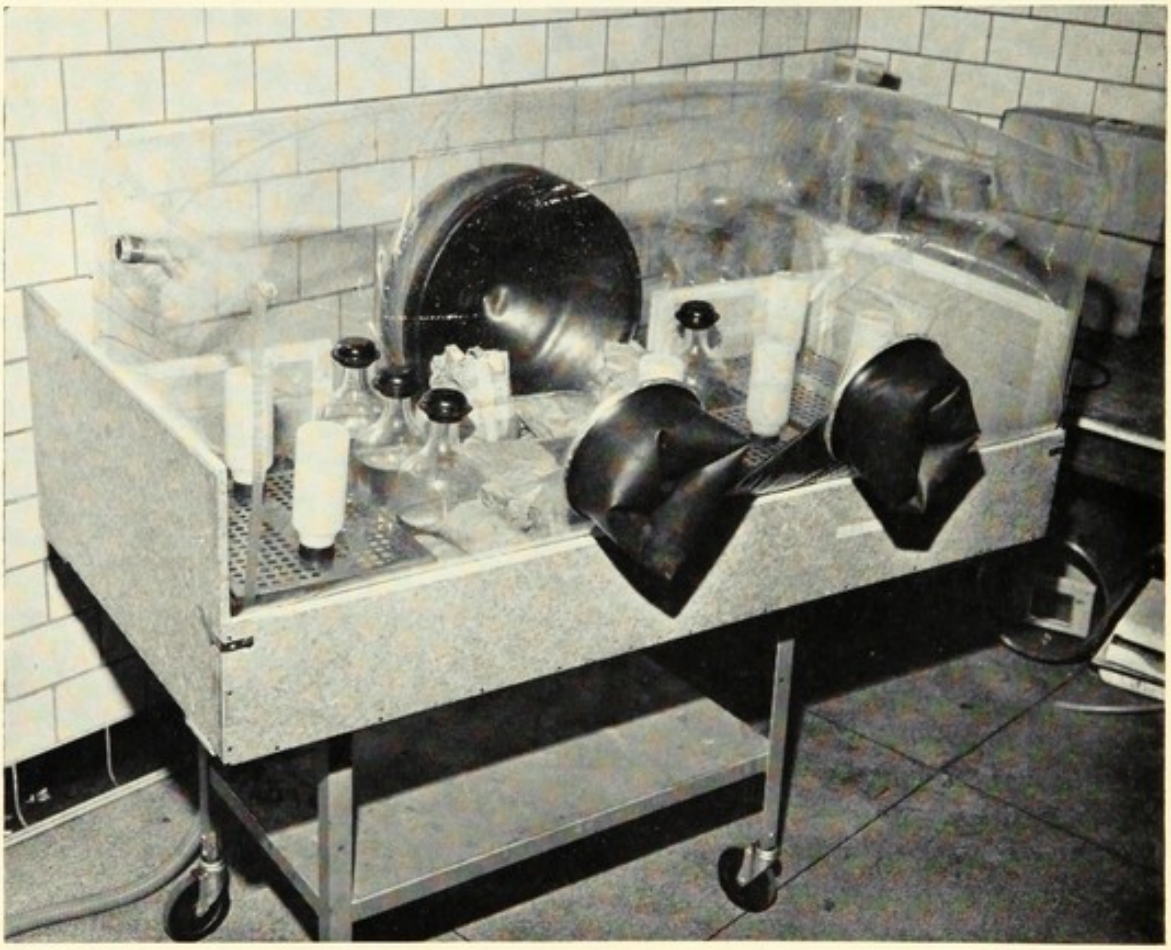

FIG. 3. Trexler Apparatus. (Courtesy of Biomed. Purview.)

autoclave entry lock. However, the autoclave increases the price considerably. Trexler (1961) has maintained animals germ-free for more than one year in this unit. The apparatus is available from G.F. Supply, Notre Dame, Indiana, as components for assembly, for a price quoted at $150.

Landy *et al.* (1961) have constructed their own polyvinyl isolator for use with germ-free pigs. It differs from the above designs in size and also in that it employs both inlet and outlet air filters. The apparatus has been used successfully and the authors stress the fact that thirty-five of these isolators are built for the price of three stainless steel isolators.

E. TRANSPORTATION UNITS

It is often necessary to transport germ-free animals either long distances from one institute to another or to some specialized apparatus, such as a source of irradiation. For this purpose, specialized apparatus has been designed, e.g., stainless steel apparatus of Parks (1958b) and the rigid apparatus of Reyniers and Sacksteder (1958). However, it is obvious that plastic units lend themselves to this purpose and Reyniers (1959) has also transported germ-free animals in glass jars fitted with free-filters.

F. DISCUSSION

The problem facing an investigator deciding to use germ-free animals is in which apparatus to invest. The guiding principles may be determined by the type of experiments envisaged. If, for example, several prototype experiments are to be carried out, the result of which will indicate whether or not to continue experimentation, there would be no justification for buying expensive stainless steel equipment, and plastic isolators are indicated. Plastic units are also indicated where special designs are necessary as these can be fabricated with relative ease. Although a possible advantage can be seen for isolators of stainless steel fitted with autoclaves where long-range experiments are concerned, it is felt by Trexler (1959, 1961), Gustafsson (1959) and Lev (1962) that the future definitely lies with plastic apparatus for the reasons discussed, and the latter two workers prefer an autoclavable plastic. If facilities are available for the construction of plastic apparatus then the savings in cost, time and design outweigh many other considerations.

III. ANIMALS

A. INTRODUCTION

The rearing of germ-free animals poses two main problems for the investigator. First generation young animals are reared apart from their conventionally reared mothers and this necessitates special care, which in the case of mammals can be a very laborious procedure. The other factor is the nutrition of the animals. The diet is sterilized, and steam has been widely used for this purpose. Destruction of vitamins and other food components results, and the investigator must ascertain that the diet in use is nutritionally inadequate for the conditions of sterilization. Moreover, bacteria comprising the gut flora, which under natural conditions can supply a certain amount of vitamins, are absent from the germ-free animal. This renders the nutritional inadequacy of the diet more acute. Reyniers *et al.* (1960) have studied the effects of steam sterilization on the content of diet L285. Thiamine HCl was most affected by heat and was decreased to $\frac{1}{3}$ of that placed in the diet. Forbes and Park (1959) have also noted the effect of steam sterilization on the thiamine content of diet and have stated that as much as 99% destruction of this vitamin occurred during their sterilization procedure. Luckey (1959) also notes that more than

95% thiamine is destroyed. This means that heat sterilized diets contain 99% of thiamine breakdown products one of which, oxythiamine, is known to be an antivitamin. Loss of other vitamins does not exceed 40-50%. The nutritional value of proteins is affected by steam, as is also the availability of lysine (Wostmann, 1959).

Many species of animals have been reared germ-free. These include monkeys, burros (donkeys) and a lamb (Trexler, 1961). Kuster (1925) raised a goat germ-free for a period of 35 days. However, only animals which have been studied in some detail and used experimentally will be discussed.

The source of conventional animals used to start experimentation is of the utmost importance. These should be obtained from healthy individuals from a reliable source; if possible, from a disease-free colony. It may be that the results of subsequent sterility tests on the germ-free progeny may show whether or not a certain source was suitable, as birds may harbour pleuropneumonia-like organisms or salmonellae may be carried over in the egg. The above considerations have led to practical procedures for rearing animals germ-free. The rearing of these animals is discussed.

B. CHICKS

The embryo of healthy birds is maintained in a germ-free condition within the shell until hatching. This has enabled germ-free chicks, turkeys and other birds to be obtained with relative ease. Germ-free chicks are very popular as experimental animals and have been successfully applied to many research problems such as the study of the origin of blood group B agglutinins (Springer *et al.*, 1959), the growth stimulation of dietary antibiotics (Lev and Forbes, 1959), and experiments on tumorigenesis (Reyniers and Sacksteder, 1959). Chicks have also formed the basis for a study of the germ-free state *per se* compared with the conventional animal (Reyniers *et al.*, 1960).

1. Egg sterilization

The most critical technique in producing germ-free chicks is that of providing adequate sterilization of the shell of the egg without injuring the embryo. Although earlier workers (Nuttal and Thierfelder, 1897; Schottelius, 1899, 1908, Cohendy, 1912) all used mercuric chloride solution to sterilize the egg, Reyniers and his colleagues were the first to perfect this technique. Reyniers *et al.* (1949a, b) conducted a series of careful experiments and employed a wide range of sterilizing agents. Their technique of washing fertile eggs with detergent and subsequent immersion in mercuric chloride has been, with minor variations, universally adopted.

Eggs from a good flock of battery-reared hens are essential; eggs which are dirty must not be used. The procedure used by the Lobund group has been changed over the years. The system described in their latest report (Reyniers

et al., 1960) involved two sterilizations. Fertile eggs on arrival from the hatchery were allowed to assume room temperature, washed under a stream of detergent (1% sodium lauryl sulphate in distilled water) and immersed in 1 or 2% mercuric chloride solution for 5 min. All solutions were maintained at 27°-28°C (80·6°-82·4°F). The eggs were then placed in a commercial incubator and turned for the first 18 days. Eggs at 20 days were candled and placed in a long narrow open-mesh nylon bag and then immersed in 1 or 2% mercuric chloride solution for 5 min at 37·5°-38°C (99·5°-100·4°F). This mercuric chloride solution formed part of the germicidal trap, which is linked directly to the germ-free unit. Immediately on immersion of the eggs in the germicide, the bag containing them is hooked on to a chain leading into the germ-free unit and which is accessible to the glove of the operator. On completion of the sterilization, the bag of eggs is carefully pulled into the germ-free unit. The eggs are removed from the bag, the germicide is allowed to dry on the surface of the eggs and the eggs are allowed to hatch. A temperature of 37·5°C is maintained in the unit with a relative humidity of 50%. Good results are reported using this technique. These authors (1949b) state that about 95% of fertile eggs are germ-free and the effectiveness of mercuric chloride in eliminating bacteria from the shell lies partly in the cleanliness of the shell and partly in the long contact period of the antiseptic with the shell. Luckey *et al.* (1960) report a hatch rate of 48% for White Leghorns and 40% for White Wyandotte Bantams.

Modifications of this sterilization procedure have been reported. Forbes and Park (1959) used 18-day embryonated eggs and immersed the eggs contained in a narrow open mesh bag for 2 min in a 0·15% (w/v) detergent (PN-700 conditioner, Service Industries, Philadelphia, Pa.). The eggs were then immersed for 10 min in a 2% mercuric chloride solution. Both detergent and antiseptic solutions were maintained at 37°C (98·6°F). The latter operation was performed in the germicidal trap leading directly to the germ-free unit. Good results were obtained using this technique; of 24 eggs set, 18-24 hatched. A temperature of 37°C was maintained in the units during hatching and this was reduced to room temperature over the period of a week.

If entry of eggs is made into a germ-free unit through a germicidal trap which does not contain mercuric chloride, a precipitate may form on the eggs, which is undesirable. This may be overcome by rinsing the sterile eggs briefly in a separate portion of the germicidal trap germicide before entry into the unit. This prevents too much precipitate forming in the germicidal trap of the unit. Although Reyniers *et al.* (1949b) stress the importance of allowing the mercuric chloride to dry on the egg shells, germ-free chicks have been obtained successfully using this method (Coates *et al.* 1963). In these experiments a hatching temperature of 37·5°C (100°F) and a relative humidity of 60% was maintained in the units. The eggs were manipulated gently during immersion in detergent and mercuric chloride, using rubber gloves. After

hatching, the egg shells, bags and dead eggs were placed in a receptacle and removed from the unit at the first opportunity. The chicks are offered diet and water.

2. Diets

Because of the destruction of thiamine during autoclaving, Forbes and Park (1959) have increased the thiamine content of their diets by ten-fold and obtained significantly better growth with diets C7 and C8, corn starch and corn soyabean meal diets respectively. The comparative growth of germ-free White Leghorn chicks on these diets is seen in Table I, adapted from the data

TABLE I

Growth of Germ-free Chicks[1]

Diet	Cl[2]	ClR[3]	C8[4]
Sex			
Male	383	403	368
Female	332	362	332

[1] Forbes and Park (1959). [2] 11 chicks. [3] 7 chicks. [4] 11 chicks.
Figures represent average weight (g) on 28th day.

of Forbes and Park (1959). Forbes and Park then compared their germ-free chicks with conventional chicks reared in their animal room (AR) and reared in a Reyniers unit (RU) in a contaminated state. This is shown in Table II.

TABLE II

Comparative Growth of Conventional and Germ-free Chicks[1]

Status	Diet	No. of Expts	No. of Chicks	Ave. wt. gain (gm)/chick[2]
Germ-free	Cl	5	40	307 ± 12[3]
Conv. in RU	Cl	3	27	240 ± 9
Conv. in AR	Cl	3	34	249 ± 113
Germ-free	C8	5	41	310 ± 7
Conv. in AR	C8	6	62	241 ± 6

[1] Forbes and Park (1959).
[2] For 28-day period.
[3] Standard error of mean.

Luckey (1959) reported growth of White Leghorn chicks reared on diet 56227 plus supplements of yeast extract and liver. None of the supplements gave enhanced growth and the weights of the chicks ranged from 213-245 g at 4 weeks. Egg production and reproduction were also obtained when 1% more minerals was added to the diet of White Wyandotte chickens. However, the second generation did not survive.

Gordon *et al.* (1957) fed diet L289F to White Leghorn chicks. This diet allowed normal growth of germ-free chicks. The birds of mixed sexes reached

a weight of 288 g (mean of 26 germ-free birds) compared to a weight of 304 g (mean of 38 conventional birds) at 35-57 days of age. Reyniers *et al.* (1960) fed White Leghorns diet L245. The chicks reached a mean weight of 350 g at 30-35 days. Luckey, *et al.* (1955) fed a practical type diet to White Leghorn and New Hampshire Red chicks. The average weight of four White Leghorn males at 4 weeks was 287 g; one female weighed 201 g.

C. TURKEYS

Although turkeys have not been widely used as experimental animals, germ-free turkeys have been successfully applied to studies on the nutritional effects of antibiotics (Forbes *et al.*, 1958) and on the infectivity of *Mycoplasma gallinarum* in relation to chronic respiratory disease (Smibert *et al.*, 1958). The same principles appertaining to germ-free chicks have been applied to turkeys by Luckey *et al.* (1960) and by Forbes and co-workers. Luckey *et al.* chose Beltsville White poults as they were small birds and so were better suited to the space available in the germ-free units, in which they could be reared up to 6-8 weeks of age.

Forbes *et al.* (1958) used Maryland Medium-White poults and Beltsville White poults. Twenty-day embryonated eggs were washed at 37°C in a solution of detergent for 2 min and then immersed in 2% mercuric chloride solution for 12 min. The eggs were introduced into a previously sterilized Reyniers germ-free unit. Twenty-four eggs were set and these yielded 14-16 poults. A purified Drackett protein starch diet was fed after autoclaving. Excellent growth was obtained on this diet; the germ-free poults grew uniformly better than conventionals. The results of two experiments are adapted from the data of Forbes *et al.* (1958) in Table III.

TABLE III

Growth of Germ-free and Conventional Turkey Poults[1]

No. of poults germ-free	Ave. wt. at 14 days (g)	No. of poults– conventional	Ave. wt. at 14 days (g)
23	202	33	170
27	201	37	170

[1] Forbes *et al.* (1958).

Luckey *et al.* (1960) have described experiments in rearing turkeys germ-free. The embryonated eggs were washed in 1% aqueous sodium lauryl sulphate, immersed in 2% aqueous mercuric chloride solution and incubated in a commercial incubator. On the 27th day of incubation, viable eggs were immersed for 5 min in 2% mercuric chloride during the introduction of the eggs into the germ-free unit where they were allowed to hatch.

The poults were fed the supplemented turkey starter mash of Pfizer & Co. (Brooklyn, N.Y.) and from their data the germ-free poults grew at a rate comparable to the conventional animals fed the same diet. A hatch rate of 64% was found, which was compared to a hatch rate of 48% for White Leghorn chickens and a hatch of 46% for White Wyandotte Bantam chicks. The general appearance of the poults was good the authors state that turkeys appear to be a satisfactory animal for germ-free research.

a. Discussion. The nutritional status of germ-free chicks and turkeys is very good. Forbes and co-workers have consistently obtained better growth of the germ-free compared with conventional chicks and turkeys. Germ-free chicks grew 15-20% better than controls. This was presented as evidence for the depression of the growth rate by bacteria in the gut. Other workers have obtained growth of germ-free birds comparable with conventionals. There was no perosis seen in chicks fed the diets quoted, in contrast to earlier diets. With the introduction of irradiated diets even better growth of chicks may be obtained (C. F. Coates *et al.* 1963).

Germ-free birds are not usually reared through more than one generation although this has been reported (Reyniers *et al.*, 1949a). This is partly due to the ease in obtaining first-generation young by sterilization of the shell and absence of a weaning period. Also, adult birds require a lot of space. The type of experiment for which germ-free chicks and turkeys are to be used is also a determining factor and short-term nutritional experiments would not merit keeping birds to the adult stage for breeding.

D. RATS

The germ-free rat has been studied in great detail by several workers and described in monographs by Reyniers *et al.* (1946) and by Gustafsson (1948). Experiments with the germ-free rat have already provided a wealth of experimental results in such fields as the etiology of dental caries (Orland *et al.*, 1955; FitzGerald *et al.*, 1960), the biosynthesis of histamine (Gustafsson *et al.*, 1957), irreversible haemorrhagic shock (Zweifach *et al.*, 1958), as well as the nutritional studies which must necessarily form a basis for applied experiments.

Gustafsson (1948) lists the advantages of the rat as an experimental germ-free animal. The nutrition of the conventional animal has been fully studied and the rat has been used extensively for metabolic experiments. Because of its small size, the germ-free apparatus need not be very large. The rat is also prolific so that experimental and control rats can be obtained from the same litter. The disadvantage with rats is the very undeveloped state at birth, requiring care in the germ-free apparatus, together with the relatively long lactation period of 21-25 days. However, the Lobund group (Trexler, 1961) and Gustafsson (1959) have reared their rats through several generations so

that the initial difficulty of weaning the germ-free animals is offset once a breeding stock is established.

1. Strains

Reyniers *et al.* (1946) used white rats of the Lobund (inbred Wistar) strain. Horton and Forbes (1958a) used C.F., Nelson and the Holtzmann Sprague-Dawley strains. Gustafsson used the Long-Evans, and Pleasants (1959) used rats of the Holtzmann, Harlan, Long-Evans and Lobund strains.

2. First-generation animals

As with all mammals, it is important to perform the caesarean operation on pregnant rats as near to the time of natural delivery as possible in order to obtain viable animals. With Gustafsson's method (1948) two female rats in heat were mated simultaneously. The onset of heat was determined by the method of Long and Evans (1922). The caesarean section was performed on the other animal after one of the rats had delivered young naturally. This indicator rat was used as a foster mother for subsequent control animals.

Reyniers *et al.* (1946) used the ear-quiver test of Griffith and Farris (1942) to select which females were on heat, and these were then placed with males overnight. The gestation period was 22 days $\pm$ 12 h. Five females selected yielded three upon which caesarean sections could be performed. Prior to the caesarean operation, the animal was given secobarbital (quinal barbitone) (35-40 mg/kg body weight). Improved techniques (Reyniers, 1957; Pleasants, 1959), allow the rat to deliver one of its young naturally before operating, and anaesthesia is avoided by killing or stunning the mother by a sharp blow to the base of the skull. Gustafsson (1948) used narcosis and cut the spinal cord at the 6th cervical segment.

After operation the baby rats are kept at an elevated temperature and humidity of 38°C (100·4°F) and 75% r.h (Horton and Forbes 1958a), or 35°C (95°F) dropping one degree every four days until weaning at the 25th day, with increased humidity (Pleasants, 1959). After detailed experimentation Gustafsson (1948) used a temperature of 37°C (98·6°F) for 24 h, 35°C (95°F) reducing to 32°C (89·6°F) over 14 days, reducing to 22°C (71·6°F) on the 21st day. A relative humidity of 70-80% was maintained over saturated sodium chloride.

3. Feeding techniques

The newly-born rats are incapable of feeding themselves and require some method of artificial feeding. Gustafsson (1948) evolved a method of filling the stomach of the baby rat in order to cut down the frequency of feeding from that required when the baby rats were allowed to suck from a nipple. The animals were fed by means of catheters with diameters of 0·5-1·0 mm depending on the age of the rats. These catheters were made by vulcanizing rubber solutions on to a stainless steel-flamed wire 0·25 mm diameter. The

catheter was attached to a glass syringe containing the milk, which was homogenized by sucking up and down repeatedly. The young rat was held by the head so that the filling of the stomach was observed. The animal was allowed to swallow the catheter until its tip reached half way between the larynx and cardia, and was arranged so that the animal could breathe relatively easily. The milk was injected slowly and the filling of the stomach was seen through the thin skin of the abdomen. When the feeding was completed the pylorus lay to the right of the midline. The catheter was slowly withdrawn to allow the oesophagus to contract.

The feeding schedule found to give the best results was as follows: the newly born rats were allowed to rest for 1 to 2 h; they were weighed and 0·05 ml of milk given. After 3 h, 0·1 ml of milk diet was given followed by 0·15 m at three-hourly intervals for the first 24 h. On the second day 0·2 ml of milk was given six times and thereafter the amount was increased by 0·2-0·25 ml per day. After the 4th or 5th day, the interval between meals was increased and the animals fed four times during the day and once at night. From the 16-17th day, small amounts of food were placed in the cage. On the 21st day feeding by catheter was discontinued and milk was given in a bowl. The animals were given solid food and water after 27-30 days. Stimulation of urination and defaecation by stroking the anal regions with a cotton plug was performed after every meal and the nostrils were cleaned at the same time. Urination and defaecation occurred spontaneously after 12-14 days.

Reyniers *et al.* (1946) used a specially made latex teat made to the shape of a rat teat. This was attached to a small pipette which was filled from the plunger end. The young were held by the head and allowed to suckle the teat. Feeding was started within three hours after birth if the animals were vigorous. Hourly feedings were begun and this required a team of at least four technicians. This regimen was continued until the 20th day. The perineal region of the animal was stroked gently after each feeding.

A forced feeding technique has been adopted by the Lobund workers and was described by Pleasants (1959). A tapered latex tube was used which blocked the oesophagus and thus prevented regurgitation into the trachea. The diet was injected quickly as the breathing was disturbed during feeding. Two or three feedings of sugar-free Tyrode's solution were given after birth. The milk diet formula L420 was given at two-hourly intervals at a rate of 0·1 ml per feed, increasing by 0·05 ml every 2 to 3 days. The proportion of butter fat in the diet was reduced from 10·8 to 7·2% after one week by combining one part cream to three parts of milk. Urination and defaecation were stimulated until the animals were 15 days old.

During the weaning period the danger of getting fluid in the lungs of the baby rats is stressed by all workers. Rupture of the oesophagus and stomach may also occur. If too much diet is given it can be regurgitated and aspirated into the trachea. Horton and Forbes (1958a) reported that death in most cases

was due to aspiration pneumonia during their experiments. Each rat had to be fed at least 150 times before it could feed without assistance. Digestion problems were also in evidence and gave rise to bloating which Gustafsson suggests may be due to milk passing undigested to the duodenum. This may have been due to overfeeding and also to the swallowing of air.

Horton and Forbes (1958a) reared three rats from ten germ-free animals started; the germ-free grew much more poorly than the mother-fed conventional controls. Pleasants (1959) reports good results on his regimen; 85% of young rats started in five experiments were weaned and nearly all females proved fertile. Cataracts which appeared in early experiments were eliminated by the addition of 0·1% methionine and 0·03% tryptophan to the liquid diet.

4. Diets

Diets are given by Gustafsson (1948, 1959) for the growth of germ-free rats. Eighteen germ-free and twenty conventional male rats fed the same autoclaved diet grew at almost equal rates reaching approximately 290 g at 120 days. Good reproduction was obtained with the diets under the conditions stated for sterilization.

The Lobund group (Wostmann, 1959) now use two diets for the post-weaning period of rats; a semi-synthetic diet (L356) and a practical diet (L462). These diets fed to Lobund male rats induced a weight gain of 1·0 g per week. However, the growth of germ-free male rats was approximately 80% of the gain of conventional rats, while with the females a slight growth retardation was noticed.

a. Discussion. The weaning period for germ-free rats requires great care and patience and survival figures for the young are rather low. Gustafsson (1948) quotes experimental results from October 1945 to February 1948. Caesarean sections were performed on twenty-two rats and seventy-eight germ-free young were delivered of which eleven (approximately 15%) were weaned and lived for 21 days or more. Horton and Forbes (1958a) delivered 477 young from seventy-three full-time pregnant rats. Twenty-three out of the 477 survived to maturity and were maintained germ-free for periods ranging from 3 to 15 months. This represents a 5% survival of useful animals. The germ-free rats had rougher fur and distended caeca compared with their conventional counterparts. These signs were also recorded by Gustafsson (1948) and the Lobund group.

Gustafsson (1948) has pointed out that the weaning weight of hand-reared germ-free rats can be increased by increasing the fat content of the diet to 20% and that the frequency of the feedings can be reduced. Lysine and tryptophan were determining factors for the numbers of animals which could be reared successfully. A high tocopherol acetate level in the milk formula is necessary also during the weaning period in order to produce fertile animals.

E. MICE

Little has been published on the detailed rearing of germ-free mice. The procedures used have been closely modelled on those used for germ-free rats. Pleasants (1959) has reared Swiss mice and the C3H strain. Two per cent novocain was injected along the midline prior to hysterectomy. Zinc was included in the mineral supplement as this has been shown to be a colostrum factor for mice (Spray, 1950). The mice were much more susceptible than germ-free rats to feeding injuries and only 6% of those delivered by caesarean section were reared. C3H mice proved difficult to rear due to a syndrome of pulmonary haemorrhage and oedema with no inflammation. This disease occurred almost entirely in the 5th and 6th days of age. Mice as well as rats were weaned at 15 days instead of the 21-25 days used in earlier experiments. Early post-weaning deaths were due to intestinal volvulus associated with caecal enlargement.

Wostmann (1959) fed diets L356 and L462 and the results did not differ substantially from those obtained with rats. The growth retardation observed was similar to that obtained with hand-reared rats.

The problems with mice are thus slightly more acute than those with rats, due to the small size and greater fragility of germ-free mice. The pulmonary syndrome described by Pleasants (1959) is analogous to the syndrome described by Phillips and Wolfe (1959) found in germ-free guinea-pigs. The possibility exists, as with other mammals, that the diets fed may not be nutritionally adequate and may contribute to the causes of enlarged caecum and other abnormalities. Better techniques in the sterilization of diets, such as sterilization by irradiation, may eliminate some untoward effects. Luckey *et al.* (1955) have shown the nutritional adequacy of a semi-synthetic diet-feed for conventional mice which was sterilized by irradiation at 2×10^6 rad. Less vitamins were destroyed during irradiation compared with steam sterilization procedure.

F. GUINEA-PIGS

The very popular use which the conventional guinea-pig has as an experimental animal has given studies with germ-free guinea-pigs special interest. Excellent studies on the application of germ-free guinea-pigs to problems of infection have been made by Phillips and Wolfe (1959) and by Formal *et al.* (1961).

An important factor in the use of guinea-pigs is the ability of the young animals to feed and look after themselves directly after birth. They do not require the feeding and care demanded by first-generation rats and mice. Against the ease of rearing young is the disadvantage of the considerably enlarged caecum of the germ-free guinea-pig which does not appear to evacuate and may lead to the death of the animal. Also, difficulty is encountered in breeding.

The guinea-pig was the first animal to be used in germ-free experimentation. Nuttal and Thierfelder (1897) raised guinea-pigs germ-free for 13 days. Cohendy and Wollman (1914) succeeded in maintaining their animals germ-free for 29 days. Glimstedt (1936) stressed the importance of nutrition in the rearing of germ-free guinea-pigs. It is thanks to the recent investigators, Reyniers, Phillips and Miyakawa, that much is known about rearing these animals germ-free.

1. Strains

N.I.H. and Hartley strains were used by Phillips *et al.* (1959). No difference could be detected between the strains, which were equally good. Horton and Forbes (1958b) used Hartley and Walter Reed strains. The Walter Reed strain was found to be less hardy than the Hartley and was less suitable for germ-free work. Miyakawa (1959) used the Gifu strain.

2. Time of delivery

Germ-free guinea-pigs are difficult to rear through more than one generation, so that obtaining first generation germ-free guinea-pigs is a routine procedure. It is of obvious importance in caesarean operations to judge the time of natural parturition as closely as possible, since premature and immature animals do not usually survive the first few days. Phillips *et al.* (1959) used several procedures to guide them as to the best time for caesarean section. The ability to express milk from the teats of the mother was not satisfactory. Radiological observations of the pubic bones were then made. Diastasis of the pubic bones two weeks before delivery is 6 mm which increases at 0·8 mm per day until 17-18 mm distance occurs. A rapid expansion to 21-22 mm occurs in the following 24 h, during which time the caesarean sections were found to be most successful. Phillips *et al.* discontinued radiological observations and relied on palpating the pubic region to determine the most appropriate time. This method was successful and used routinely. Miyakawa (1958) also used palpation to estimate approaching parturition and stated that the tip of the thumb can be inserted between the relaxed symphysis 24-36 h before delivery. The pelvic muscles relax and the pelvic area becomes oedematous a few hours before delivery, at which time animals were taken for caesarean section.

3. Operation

Phillips *et al.* (1959) used the Reyniers operating unit and followed the procedure laid down by Reyniers *et al.* (1946). Horton and Forbes (1958b) describe in detail a modified procedure. The abdomen of the pregnant guinea-pig is shaved and the animal is tied down and given pentobarbitone sodium intramuscularly, 130 mg per kg body weight. The abdominal surface is treated with a depilatory, scrubbed with a surgical soap solution and washed. The abdomen is soaked for 5 min with 0·1% benzalkonium

chloride solution and rinsed with sterile water. The animal is placed in the operating unit and its abdomen is rinsed with alcoholic-iodine solution (alcohol: water: Wescodyne, 70 : 20 : 10) and raised until the abdomen comes into contact with the plastic sheet dividing the upper (sterile) from the lower section of the operating unit. The operator makes a median incision through the plastic with an electric cautery. The catuery serves to adhere the plastic to the skin of the animal so that no skin surface is exposed to the interior of the upper (sterile) section of the germ-free unit.

The body wall is opened and the uterus is lifted out entire. The young animals are removed, the umbilical cords being sealed with the cautery, and handed to an assistant who cleans and revives them. They are then passed into a rearing unit. As mentioned previously (p. 142) several caesarean sections can be performed in the same surgical unit by placing a fresh sterile sheet of plastic over the operating area before removing the hysterectomized animal. The animal is then removed and the procedure repeated. The authors report no contamination using this procedure in experiments repeated over a period of 18 months.

In the operating procedure described by Miyakawa *et al.* (1958) the hair is removed from the abdomen of a pregnant guinea-pig and the abdomen is immersed in 5% lysol soap solution and washed with sterile water. The abdomen is scrubbed with a 1% solution of benzalkonium chloride for 10 min and dried. The animal is exposed for 5 min under an ultraviolet lamp, placed in the operating unit (essentially the same as the Reyniers operating unit) and raised until the abdomen of the animal comes into contact with a sheet of nylon. The skin is incised through the nylon and the muscle and fascia are soaked in detergent. The abdominal wall and uterus are opened and the amniotic membrane removed from the young. The young are transferred to a rearing unit.

4. Rearing

Following caesarean section, Phillips *et al.* (1959) placed three animals together in small cages until they were 5 days old. The animals did not develop an appetite if caged separately at an early age and also their ability to defaecate was impaired. After 5 days the guinea-pigs were caged separately until they reached the age of 40 days. At this age the anal sucking habit—common in younger animals housed together and resulting in anal prolapse—was not found. A hair-eating tendency, also common in young animals, usually did not develop after 60 days of age. The animal developing hair-eating after this age was segregated or destroyed before damaging the others. As the guinea-pigs ingested large amounts of bedding material, muslin was used as bedding material for animals less than 8-10 weeks of age. This did not tend to occlude the alimentary canal as did wood shavings. Older animals were housed on wood shavings. A temperature of 32-33°C (89·6-91·4°F) was maintained in

the germ-free units for the first 7 days, 31°C (87·8°F) for 3 weeks and 30°C (86°F) for the following 6 months. Older animals were reared at 28°C (82·4°F). Miyakawa *et al.* (1958) used lower temperatures, starting at 30°C and falling to 25°C (77°F) after one week. Horton and Hickey (1961) employed essentially the same system as Phillips *et al.* (1959).

5. Nutrition

The nutrition of the germ-free guinea-pig is not at a stage comparable with, for example, that of the germ-free chick, where better growth of the germ-free animal compared with the conventional animal is usually obtained. This can be seen by the slower growth rate and by symptoms such as poor hair growth and prolapsed anus. The enlarged caecum may also be an indication of dietary insufficiency. That bacteria of the gut flora supply some nutritional factors can be seen from the inability of germ-free guinea-pigs to grow on a supplemented commercial ration while their conventional counterparts grow well on this sterile diet. Most workers have described a dietary regimen consisting of a liquid diet for its young animals followed by a solid diet. The results of the most successful diets used by different workers are discussed.

Miyakawa *et al.* (1958) used a liquid diet NG27 for the first 5 days, and then a mixture of NG27 and NG36 (Kobayashi) solid diet was given until the animals were a month old, after which only the solid diet was fed. Miyakawa stated that the liquid diet is not satisfactory but that the solid diet is suitable for long-term experiments. His guinea-pigs reached a weight of approximately 300 g at 40 weeks and gained approximately 1 g per day.

Phillips *et al.* (1959) have made extensive studies on the nutrition of the germ-free guinea-pig. Their semi-solid diet L445, which is a Quaker Oats, chow dextrose, mixture supplemented with vitamins, was their best diet for rearing germ-free guinea-pigs. Although very little diet is consumed in the first 48 h, the authors did not consider this important and after 8 days animals showed an increase over the birth weight, gaining an average of 3 g per day. This can be compared to the average of 1 g per day obtained by Miyakawa *et al.* (1958). Twenty per cent mortality occurred in the animals of Phillips *et al.* (1959) on diet L445. Horton and Forbes (1958b) used this diet with the exception that the ascorbic acid was sterilized by irradiation at 2×10^6 rad. The animals were given 5 mg ascorbic acid and 100 mg thiamine HCl per animal per day. Fresh diet from a container was given twice daily. The germ-free and conventional guinea-pigs grew at an equal rate and reached a weight of 500 g at 15 weeks.

An important contribution to germ-free guinea-pig nutrition is the irradiation-sterilized diet of Horton and Hickey (1961). All previous diets had been sterilized by autoclaving. The semi-synthetic diet (No. 17) was irradiated at 2×10^6 rad and the sterilizing procedure was monitored with *Bacillus subtilis* spores. A liquid form consisting of three parts of water to one part of

diet was fed for the first 3 weeks together with the dry form of diet. Fresh liquid diet was offered twice daily. Dry diet only was given after 3 weeks.

Good results were obtained using this diet and the average weight of six males and five females was 500 g at 15 weeks. Animals as small as 67 g at birth did well. The animals showed a daily weight gain of approximately 4 g. An interesting point of this study was that the caeca in the germ-free guinea-pigs were no bigger than in the conventional animals, although they were very thin and lacked tonus.

6. Disease

In addition to deficiency symptoms obviously due to nutritional inadequacy, several workers have described lesions in germ-free guinea-pigs which may be due to infectious disease. Miyakawa *et al.* (1958), Horton and Forbes (1958b) noted a pneumonic disease in germ-free guinea-pigs which was attributed to a virus. This disease occurred and disappeared spontaneously and was studied in detail by Phillips and Wolfe (1961), when it appeared in young animals from consecutive caesarean sections from sixty females. The disease occurred when the animals were 10-14 days old and was invariably fatal, death being due to an acute bronchopneumonia. Prophylaxis by elevating vitamin levels or by administering antibiotics had no effect on the outcome of the disease. The only procedure which prevented symptoms was the feeding of autoclaved caecal contents of conventional guinea-pigs to germ-free animals for the first 5 days. The authors conclude that the disease was due to a virus which was transmitted transplacentally and that the germ-free animal is susceptible since conventional animals do not contract the disease. The consideration remains that the autoclaved caecal contents may have supplied an essential metabolite. The germ-free stock was thereafter selected from a disease-free colony and no recurrence of the pneumonic disease was seen.

The germ-free guinea-pig has been studied in some detail and has proved itself not only a useful experimental animal for the study of infectious processes but also one from which the knowledge of the nutrition and physiology of the animal can be obtained and the contribution of the intestinal flora ascertained. Diets sterilized by irradiation have shown the best results.

G. PIGS (MINIATURE STRAIN)

The general techniques laid down for obtaining and rearing mammals germ-free have been successfully applied to the pig by Landy *et al.* (1961). Their own design of a peracetic acid-sterilized plastic unit was used. These authors stress the need for large germ-free animals in germ-free research. The pig seems to be well suited for this purpose, as at birth the eyes are open and the animal can walk about and feed itself within a few hours of delivery. There is usually a large litter which can be divided between germ-free and

control groups, or lends itself to other arrangements. The gestation period is uniform enough to permit operative delivery with a high yield of viable animals. There are also close similarities to man in the physiology and anatomy of the skin, vascular system, eye and gastro-intestinal tract. In addition, the pig has been used extensively in nutritional studies and is omnivorous. Germ-free pigs have been used in a study of bile peritonitis.

Germ-free pigs were derived from miniature gilts obtained from the Hormel Institute, Austin, Minnesota.

1. Operation

Pregnant gilts were delivered on the 109-111th day after mating. The gilts were about a year old and weighed approximately 175 lb. The abdomen of the gilt was carefully shaved and scrubbed with hexachlorophene soap. Three coat of 7% aqueous iodine were applied and each coat allowed to dry. The fully sh of the plastic germ-free operating unit was attached to the abdomen of the supine animal with a sterile adhesive and 11 mg per kg thiamylal sodium were injected into the anterior vena cava *via* a spinal needle. A cautery was used to incise through the plastic and the skin. This killed any organisms present in hair follicles. The laparotomy was completed with a scalpel and the uterine horns entered manually. The piglets were handed to an assistant who completed the removal of the membrane and clamped the umbilical cords. Each animal was rapidly passed through a sleeve connection to another sterile unit where assistants dried the coat, aspirated the pharynx, tied the umbilicus with cotton tape and applied artificial respiration when necessary. The deliveries were completed within 3 min.

2. Feeding schedule

The new-born piglets accepted food 2 to 6 h after birth and approximately 80% were able to drink immediately from a bowl or trough. The remainder were fed from a bottle, although an occasional piglet needed feeding for 2 weeks. The piglets were fed three times a day—7 a.m., noon, and 4 p.m. for 5 to 10 days, after which the noon feeding was omitted. For the first five days 125 m of diet were fed at each of the three meals and this was increased stepwise to 250, 500, 750, 1,000 and 1,500 m twice a day. Thus the animals were given 2,000 m per day at 5 weeks of age and 3,000 m at approximately 6 weeks of age.

The temperature of the rearing unit is initially maintained at 32°C (89·6°F) with a r.h. of approximately 50%. The temperature is subsequently lowered to 25°C (77°F) over a period of 1-2 weeks.

3. Diet

The diet Landy *et al.* used was homogenized cow's milk supplemented with vitamins and minerals, which was autoclaved at 118-120°C (244·4-248°F) for 20 min. Thermocouples were used to monitor the sterilization

cycle in sample bottles of milk as caramelization and coagulation occurred with too high a temperature, and inadequate sterilization was a hazard at too low a temperature. On cooling, the milk bottles were placed in a plastic unit and surface sterilized by spraying with peracetic acid. Sterility tests were taken from samples of milk and the surface of the unit. If negative, the unit received a germ-free piglet. Other workers (Tennant, 1961) have used a commercial bitch's milk substitute (Esbilec-Borden Co., New York, N.Y.) supplemented with vitamins.

Landy (1961) reports the results of a comparison of the growth of twenty-three germ-free and 352 conventional pigs at the Hormel Institute. The latter animals were allowed to suckle and had their diet supplemented with pig chow. Although the weight of the germ-free piglets at birth was little over a pound compared to a weight of two pounds for the conventional piglets, the germ-free animals at six weeks weighed on average one and a half pounds more than the controls (13 : 11½ lb). The germ-free animals reached 10 and 15 lb weight, 3 and 8 days earlier than Hormel conventional pigs and also required little care and attention. These results which show that germ-free pigs are reared with relative ease with none of the complications seen with rats, guinea-pigs and mice, are of great interest, and would indicate a definite place for pigs in germ-free animal research.

H. RABBITS

Germ-free rabbits have been reared with limited success. The problems lie in the nutrition of the weaned animal and so far a satisfactory diet has not been described.

Pleasants (1959) reports on the weaning period for germ-free rabbits. Local anaesthesia, 2% procaine hydrochloride, injected along the midline, was used prior to hysterectomy. A gestation period of 30 or if possible 31 days was chosen. Temperatures were set 1° or 2°C lower than for rats and mice. The baby rabbits were allowed to suck from a rubber nipple every two hours and the amount fed per feed varied from 1 ml on the first day to 5-6 ml on the 21st day. The rabbits tended to overfeed and restriction of intake was necessary. Diet L-449G-E1 was fed. This contained choline and cystine which prevented abnormally fatty livers in hand-reared rabbits. Urination and defaecation were stimulated by stroking the appropriate regions, although defaecation was found to occur spontaneously.

Good results are reported for the weaning period. The weights of the young germ-free animals were closer to conventional mother-fed animals than is the case with hand-reared rats and mice.

Wostmann (1959) fed weaned rabbits on Purina Rabbit Chow plus 10% casein, two parts of which were added to one part of bran and vitamins. Although the steam-sterilized diet gave good results with conventional animals, most germ-free animals died after a few months. The caeca were

very distended, muscular wasting and renal cortical nephritis also occurred. Vitamin E and choline were present in adequate amounts in the diet as well as a suitable balance of Ca, P and Mg. Another diet based on a diet for germ-free guinea-pigs gave no better results.

The poor state of nutrition of the germ-free rabbit at present precludes its use as an experimental animal. The results with germ-free rabbits are reminiscent of the early attempts in rearing germ-free guinea-pigs. Diets sterilized by irradiation may give better results and the fact that conventional animals grew well on a diet which is inadequate for germ-free rabbits may indicate that unknown factors of bacterial origin are essential for the rabbit.

IV. Bacteriological Control

Ideally the germ-free animal is an animal reared free from other living agents, bacteria, fungi, viruses, protozoa, worms, etc. In practice bacteria and fungi represent the greatest contamination hazards. The definition of the germ-free state is limited by the tests used for detecting contaminants. Thus Gustafsson (1948) and Forbes and co-workers have limited the definition of their germ-free animals to "free from cultivable bacteria and fungi", which was covered by the tests employed.

Bacteriological control is a procedure which must be routine and yet be broad enough to cover all known cultivable bacteria and fungi. These organisms may be aerobic or anaerobic, fastidious or inhibited by complex media, and they may only grow at certain temperatures.

The determination of germ-free status has been fully discussed by Reyniers *et al.* (1949) and by Wagner (1959), and other groups of workers have adopted their own preference in media and growth conditions.

A. CULTURAL PROCEDURES

In general, tubes of culture media and swabs are introduced into the germ-free unit. The swabs are moistened and samples of anal contents, faeces, diet and skin are taken and inoculated into the media. Additional swabs are taken for inoculation on media outside the germ-free unit. A scheme for routine sterility testing is shown in Table IV. The items shown in parenthesis are not commonly used.

Table IV

Routine Sterility Test for Germ-free Units

Sample source	Anus, faeces, diet, bedding, surface of unit
Liquid media	Tryptricase soy broth or brain-heart infusion broth, thioglycollate media
Solid media (aerobic and anaerobic)	Trypticase soy agar with and without 5% horse blood
Solid media aerobic	Sabouraud's agar or potato-dextrose agar
Incubation temperature	Room temperature 22-24°C, 37°C (55°C)
Incubation time	1-2 weeks
Microscopic examination	Gram stain (wet mounts)
Interval of testing	Once weekly

Growth in the tubes of liquid media which are inoculated and plugged inside the germ-free units represent contamination even though growth may be sporadic. Sparse growth on plates inoculated outside may have come from the laboratory atmosphere and the sterility test should be repeated before concluding that contamination has occurred. In addition to cultural tests, smears of the anal contents and faeces are stained and examined. Small numbers of bacteria can always be seen in these smears and depend on and are derived from the type of diet. Wagner (1959) has amply demonstrated that killed bacteria retain their morphology after passing through the alimentary tract of animals. A rise in number of bacteria seen in smears is strong indication of contamination even though the organisms may not grow on culture. Wagner (1959) quotes an example of an organism which was detected by the relatively large numbers seen in faecal smears, and growth occurred only occasionally in thioglycollate medium. This organism showed a predilection for the caecum of the rat.

Bacteria can contaminate germ-free animals without necessarily showing an increase in numbers in smears (Lev, 1961). A gram-positive bacillus was isolated from previously germ-free chicks and was poorly adapted to growth in the gut as only one of four tubes of tryptricase soy broth showed growth. After three weeks of adaptation numbers increased, and growth was obtained on all media. Organisms which are never found in the gut of conventional animals will adapt themselves to the excellent medium of the germ-free gut and quickly spread to all animals in a particular unit.

B. TRACING OF CONTAMINATION

It is advantageous to ascertain as far as possible the cause of breakdown in a germ-free system and study of the contaminating organism may often give a clue. Reyniers *et al.* (1946) have discussed the correlation of type of organism with the type of fault leading to contamination of germ-free chickens. Organisms of faecal origin such as *Streptococcus faecalis* or *Escherichia coli* occurring as contaminants shortly after the experiment began would indicate that the shells of the eggs had been inadequately sterilized. *Staphylococcus aureus* and *Staph. epidermidis*, organisms associated with human skin, occurred after glove breaks. Moulds were attributed to a faulty filter which had become moist, allowing growth of the mould through the filter. Autoclaving technique was assumed to be the cause of contamination due to *Bacillus subtilis*; other contaminants could not be placed in relation to any special mechanical failure of the apparatus or technique.

Contamination may become apparent only some time after the organism has penetrated into the germ-free unit, depending to some extent on the particular environment upon which it settles and the adaptation of the organism to grow in the intestine, faeces or diet. This may be a disadvantage in detecting the cause of failure.

It should be stressed that success or failure of a germ-free experiment depends on small points in technique and any exposures to conventional atmosphere such as occurs, during usual bacteriological procedures, are to be deprecated. The time and expense involved in germ-free research automatically demands that only stringent technique is permissible; all autoclavings should be temperature recorded, only surface sterile objects entered through germicidal traps, and care must be taken to ensure that gloves cannot be torn on sharp surfaces, or otherwise damaged.

The main causes of contamination are bacterial and fungal; the possibility exists that contamination by worms or protozoa could occur. Phillips (1960) has conducted a parasitological survey of Lobund germ-free animals and examined mice, rats, guinea-pigs, chickens and dogs. No parasites were found in any animal with the exception of the dog which carried nematode worms although bacteria-free. Five out of six of the dogs examined carried larval or adult stages of *Toxocara canis* and one animal also carried *Ancylostoma caninum*. Most dogs were infected with nematodes which infect the young *in utero*. Phillips believes that it is possible to maintain animals prior to caesarean section under conditions to prevent nematode infection, and this could result in germ-free dogs.

C. DISCUSSION AND SUMMARY

In summary it may be said that it is incumbent on every worker in the germ-free field to employ adequate bacteriological control in the animals used. These should follow those laid down by previous workers and proved to be trustworthy. In addition, parasites should be looked for and efforts must be made to determine if pleuropneumonia-like organisms are present. Microscopic examination is of importance in detecting bacteria and other parasites which may not grow on the media. The reader is referred to the paper of Wagner (1959) and to the discussion of the Symposium on Germfree Animals (Recent Progress in Microbiology 1959, Charles C. Thomas, Springfield, Illinois) for a full treatment of the factors involved in the detection of viruses in germ-free animals.

However, the presence of latent viruses in germ-free animals is still a matter of speculation and experiments on their detection are a problem for the specialist.

Appendix

DIETS FOR GERM-FREE CHICKS

Diet L289F[1]

Yellow corn	50%
Soybean meal	23%
Wheat midlings	9%
Casein	7%
Alfalfa	2%
Fish meal	2%
Meat scraps	2%
Minerals	4%
Vitamins and carriers	1%

[1] Gordon, H. A., Wagner, M. and Wostmann, B. S. (1957-1958). Studies on conventional and germfree chickens treated orally with antibiotics. *Antibiotics Annual*, Med. Encyclopedia, Inc., N.Y. (Sterilized by steam at 121°C/25 min.)

COMPOSITION OF DIETS[1]

Constituents	*C-7*	*C-8*
Soybean oil meal, g		35·00
Yellow corn, g		58·20
Cornstarch, g	58·25	1·6[2]
Casein (purified), g	25·0	
Corn oil, g	5·0	0·5
Alphacel, g	3·0	
Glycine, g	1·5	
L-Arginine-HCl, g	1·0	
DL-Methionine, g	0·5	
Choline . HCl, g	0·27	0·27
Thiamine . HCl, g	0·025	0·1
Ca pantothenate, mg	10·0	10·0
Nicotinic acid, mg	10·0	10·0
Riboflavine, mg	4·0	4·0
Pyridoxine HCl, mg	2·0	2·0
Folic acid, mg	1·0	1·0
Vitamin B_{12}, mg	0·005	0·005
Biotin, mg	0·1	0·1
Menadione, mg	0·8	0·8
Vitamin A, I.U.	2,600	2,600
Vitamin D_3, ICU	100	100
α-Tocopherol, mg	5	5
$CaCO_3$, g		1·6
Na_2HPO_4, g	0·5	
$Ca_3(PO_4)_2$, g	3·16	
KCl, g	0·7	
NaCl, g	0·6	0·5
$MgSO_4 . 7H_2O$, g	0·4	
$MnSO_4 . H_2O$, mg	40	30
$FeSO_4$, mg	12	8
$CuSO_4$, mg	1	0·8
$CoCO_3$, mg	1	0·5
$ZnSO_4 . 7H_2O$, mg	1	1
KI, mg	0·3	0·15
$CaHPO_4 . 7H_2O$, g		2·2

[1] Forbes, M. and Park, J. T. (1959). (Sterilization by steam at 252-255°F/25 min.)
[2] Used as mix for B vitamins.

COMPOSITION OF DIETS (g)[1]

Constituents		*56227*[2]	
Salts L-II[3]	50	Choline Cl	2·0
Starch, corn	549	Ca pantothenate	0·044
β-Lactose	0·9	Pyridoxine Cl	0·012
Oil, corn	43	Biotin	0·0004
Casein, Labco	250	Folic acid	0·005
Gelatin	100	Inositol	1·0
L-Cystine	3	Vitamin D_3 (I.U.)	5,000
Vitamin A (I.U.)	50,000	Vitamin E	2
Thiamine HCl	0·016	Vitamin K	0·05
Riboflavine	0·016	*p*-Aminobenzoic acid	0·1
Nicotinamide	0·05		

[1] Luckey, T. D. (1959). (Sterilization by steam at 17 lb/20 min.)
[2] This diet, obtained from Parke, Davis & Co., Detroit, Michigan, used lard in place of corn oil and the Jones and Foster salt mix with 0·1% $MnSo_4 . 4H_2O$.
[3] The composition of salts L-11 in g/kg of diet is: $CaCO_3$, 15·0; $CaHPO_4$, 2·75; K_2HPO_4, 11·3; Na_2HPO_4, 10·0; NaCl, 2·5; KI, ·0375; $MgSO_4 . 7H_2O$, 3·5; $MnSO_4 . 4H_2O$, 0·625; $Fe(C_6H_5O_7)_2$, 3·75; $CuSO_4 . 5H_2O$, 0·19; $CoCl_2 . 6H_2O$, 0·025; $ZnSO_4 . 7H_2O$, 0·05; $Na_2B_4O_7 . 10H_2O$, 0·025; and $AlK(SO_4)_2 . 10H_2O$, 0·0375.

DIET L 445[1]

Constituents	
Purina Lab Chow	100
Quaker oats	100
Dextrose	10
Sodium chloride	5
Water	1000

Supplemented with 1 ml per animal per day

Abscorbic acid	1
Thiamine	0·05
Water	100

[1] Phillips, B. P. *et al.* (1959). (Sterilized by steam at 250°F for 20 min.)

COMPOSITION OF DIET NO. 17[1]

Constituents	*g per kg*	*Constituents*	*mg per kg*
Casein (vitamin-free)	262	Thiamine HCl	32
Quaker oats	230	Riboflavine	32
Cornstarch	118	Pyridoxine	32
Dextrose	100	Calcium pantothenate	80
Cellophane spangles	100	Niacin	200
Corn Oil	70	Biotin	1·2
L-Arginine	10	Folic acid	20
DL-Methionine	5	Vitamin B_{12}	0·08
Glycine	5	Menadione	4
Potassium acetate	25	α-Tocopherol acetate	40
Magnesium oxide	5	Natola[3]	600
Salt mixture—W[2]	60		
Choline chloride	2		
Inositol	2		
Ascorbic acid	4		

[1] Horton, R. E. and Hickey, J. S. (1961). (Sterilized by irradiation at 2×10^6 rad.).
[2] Wesson's modification of Osborne and Mendel Salt Mixture; supplied by Nutritional Biochemicals Corp., Cleveland, Ohio.
[3] Obtained from Parke, Davis & Co., Detroit, Michigan. (One gram contains 55,000 U.S.P. units of Vitamin A and 11,000 U.S.P. units of Vitamin D.)

COMPOSITION OF NG—36 DIET (KOBAYASHI) PER 100 G OF RATION[1]

Constituents		*Constituents*	
Roasted soy bean flour	70·0 g	Yeast-extract	0·5 g
Sucrose	9·0 g	Liver-extract	25·0 mg
Gum arabic	12·0 g	Inositol	100·0 mg
Salts mix	7·0 g	Pantothenic acid—Ca	10·0 mg
Panvitan (vitamin complex)	1 pellet	PABA	5·0 mg
Folic acid	2·5 mg	Thiamine	9·0 mg
Vitamin K	2·5 mg	Riboflavine	2·0 mg
Vitamin B_{12}	15·0γ	Pyridoxine	2·0 mg
Biotin	100·0γ	Vitamin C	25 mg/2 days
Choline	0·3 g		

[1] Miyakawa, M. *et al.* (1958). (Sterilization at 120°C/30 min.)

COMPOSITION OF NG—27 DIET (KOBAYASHI) PER 300 G OF RATION[1]

Constituents	
Whole dry milk	100 g
Aq. dest.	200 ml
Soy bean oil	1·5 ml
Panvitan (vitamin complex)	3 pellets
Folic acid	7·5 mg
Vitamin K	7·5 mg
Vitamin B_{12}	30·0 μg
Biotin	300·0 μg
Choline	0·15 g
Yeast-extract	1·5 g
Liver-extract	75·0 g
Inositol	300·0 mg
PABA	15·0 mg
Thiamine	27·0 mg
Riboflavine	6·0 mg
Pyridoxine	6·0 mg
Vitamin C	20-25 mg/2 days

[1] Miyakawa, M. *et al.* (1958). (Sterilized with steam at 120°C/30 min.)

COMPOSITION OF PRACTICAL RAT DIET L462[1]

Constituents	*Amt*/110 *g*
Distilled H_2O[2]	15 cc
Whole wheat flour	30 g
Corn meal, yellow	32 g
Lactalbumin, Sheffield	10 g
Casein, Sheffield new process	5 g
Whole milk powder	10 g
Alfalfa leaf meal	2 g
Desiccated liver	2 g
$CaCO_3$	0·5 g

[1] Wostmann, B. S. (1959).
[2] Added to facilitate pelleting.

B mix-75

Thiamin hydrochloride	3 mg
Riboflavine	1·5 mg
Nicotinamide	2·5 mg
Nicotinic acid	2·5 mg
Calcium pantothenate	15·0 mg
Choline chloride	100·0 mg
Pyridoxine hydrochloride	1·0 mg
Pyridoxamine dihydrochloride	0·2 mg
Biotin	0·05 mg
Folic acid	0·5 mg
Para-aminobenzoic acid	2·5 mg
0·1% trituration B_{12} in mannitol	12·5 mg
Cornstarch	108·75 mg

Ladek-36

Vitamin A concentrate, natural ester form	800 I.U.
Vitamin D_3[1]	100 I.U.
Vitamin E, mixed tocopherols	37·5 mg
Vitamin K_3, menadione	10·0 mg
Corn oil	1·958 g

Salts-17

NaCl	299 mg
KI	4·5 mg
$MgSO_4 \cdot 7H_2O$	449 mg
$MnSO_4 . H_2O$	57·7 mg
$Fe(C_6H_5O_7)_2$	449 mg
$CuSO_4 . 5H_2O$	22·4 mg
$CoCl_2 . 6H_2O$	3 mg
$ZnSO_4 . H_2O$	3·8 mg
NaF	1·5 mg
$Na_2B_4O_7 . 10H_2O$	3 mg
$AlK(SO_4)_2 . 12H_2O$	4·5 mg
MoO_3	3 mg

Analysis[2]

N × 6·25	24%
Fat	8%
Ash	3·5%

[1] Delsterol, produced by E. F. du Pont de Nemours & Co., Inc., Wilmington, Delaware.
[2] After pelleting and steam sterilization at 123°C/25 min.

DIET D5[1]

Constituents

Casein	22%	Riboflavine	2 mg
Wheat starch	63%	Pyridoxin	2 mg
Arachis oil	10%	Calcium panthothenate	10 mg
Salt mixture HMW[2]	4%	Nicotinamide	20 mg
Vitamin mixtures	1%	Choline	200 mg
Vitamins added per 100 g diet		Inositol	100 mg
Vitamin A	2,100 I.U.	P-aminobenzoic acid	30 mg
Vitamin D	450 I.U.	Biotin	0·1 mg
Vitamin E	50 mg	Folic acid	2 mg
Vitamin K	10 mg	Vitamin B_{12}	0·002 mg
Thiamine	5 mg	Ascorbic acid	100 mg

[1] Gustafsson, B. E. and Laurel, C. (1958).
[2] According to Hubbell, R. B. *et al.* (1937). (Sterilized by steam at 121°C/20 min.)

DIET L420—MILK FORMULAS AS PREPARED FOR AUTOCLAVING[1]

Constituents	
Cow's milk, whole	50 ml
Light cream (18%)	50 ml
Vi-Syneral[2]	0·1 ml
Mixed tocopherols (253 I.U. vitamin E/g)	1·7 mg
Vitamin K (menadione)	0·17 mg

[1] Pleasants, J. R. (1959). (Sterilized by steam at 120°C/15 min.)
[*] Vi-Syneral aqueous vitamin drops (U.S. Vitamin Corp. New York, N.Y.) is listed on the label as containing the following per 0·6 ml: vitamin A (natural), 5,000 U.S.P. units; vitamin D (natural), 1,000 U.S.P. units; ascorbic acid, 50·0 mg; thiamine HCl, 1·0 mg; riboflavin, 0·4 mg; pyridoxine HCl, 0·3 mg; niacinamide, 5·0 mg; pantothenic acid, 2·0 mg; (made soluble by sorethytan esters).

BASAL DIET (FOR GERM-FREE TURKEYS)[1]

Constituents	*Per 100 g diet*
Cornstarch, g	41·40
Washed protein[2]	44·44
Corn oil	4·04
DL-methionine	0·71
Glycine	0·51
Choline chloride	0·46
Diphenyl-p-phenylene-diamine	0·0125
Minerals[3]	7·45
Vitamins[4]	0·89

[1] Forbes, M. *et al.* (1958). (Sterilized at 252-255°F/25 min.)
[2] Drackett C:1 Assay protein, washed once with tap water and three times with distilled water at pH 4·6, then dried at 50-60°C.
[3] Minerals, g per 100 g diet (all c.p. or reagent grade): 1·846 $CaCO_3$, 3·313 $Ca_3(PO_4)_2$, 0·808 K_2HPO_4, 0·002 $CuSO_4 . 5H_2O$, 0·031 $MnSO_4 . H_2O$, 0·002 $CoSO_4 . 7H_2O$, 0·004 KI, 0·01 K Al $(SO_4)_2 . 12H_2O$, 0·001 $Na_2MoO_4 . 2H_2O$, 0·005 $Na_2SiO_3 . 9H_2O$, 0·001 HBO_3, 0·012 $ZnCl_2$, 0·303 KCl, 0·455 $MgSO_4 . 7H_2O$, 0·606 NaCl, 0·051 $FeSO_4 . 7H_2O$.
[4] Vitamins, mg per 100 g diet: 12 α-tocopherol acetate (from Myva-mix), 2 menadione, 60 thiamine, 10 riboflavin, 32 calcium pantothenate, 40 niacin, 6 pyridoxine HCl, 0·2 biotin, 2·4 folacin, 0·012 cyanocobalamine, 101 inositol, 1·0 p-amino-benzoic acid, 40 ascorbic acid. Also 5656 I.U. Vitamin A (from Nopcay "20") and 529 I.C.U. Vitamin D (from Nopdex "15").

COMPOSITION OF RABBIT DIET L-461B-E1[1]

Constituents	*Amt./100 g*
Purina rabbit chow, ground	62·16 g
Casein, Sheffield new process	7·0 g
Ground bran	30·0 g
BC mix-45	
Ascorbic acid	35·0 mg
Inositol	35·0 mg
Riboflavine	0·7 mg
Calcium pantothenate	3·5 mg
Niacinamide	3·5 mg
Pyridoxine hydrochloride	0·7 mg
Biotin	0·0175 mg
Folic acid	0·7 mg
Thiamine hydrochloride	1·75 mg
Vitamin B_{12}, 0·1% in mannitol	0·7 mg
Choline chloride	70·0 mg
Cornstarch	198·43 mg

[1] Wostmann, B. S. (1959).

Ladek-3

Vitamin A concentrate, natural ester form	280 I.U.
Vitamin D[1]	35 I.U.
Mixed tocopherols (1 gm=243 I.U.)	52·5 mg
Menadione	3·5 mg
Corn oil	700 mg

Mineral Composition (calculated)

Ca	980 mg
P	780 mg
Mg	290 mg

[1] Delsterol

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Chapter 6

Inheritance of Coat Colour in Laboratory Rodents

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I. Introduction

The inheritance of coat colour in laboratory rodents is a complex subject. In the mouse alone nearly sixty genes affecting the pigmentation of the hair have already been discovered. About one-third of these have not yet been located, but the others are known to occupy twenty-five distinct loci. Most of these genes interact with one another. Of the vast number of combinations possible only a few have been synthesized and described. As the differences between various genotypes are often subtle, the descriptions are not always adequate.

In the following survey the mouse has been treated as the representative of the group, and detailed accounts of most phenotypes are to be found in the section on this animal. Several colour genes have had to be left out of this section because they have so far been reported only in the *Mouse News Letter*, and quotations from that publication are not allowed. References have been given sparingly: they are intended to bring up to date the comprehensive bibliographies of Grüneberg (1952) and Little (1958). To make the reader's task easier, a brief section on the structure of the hair and its pigments has

been included. For the sake of convenience the term "colour genes" has been used for all the hereditary factors that affect pigmentation of the hair either generally or locally, regardless of whether they have any other effects. Gene symbols and linkage groups (if known) are usually given in brackets where the name of the gene first occurs.

II. Hair Structure and Hair Pigments

The rodent coat consists of three chief types of hairs. They are, in the order of their size, guard hairs, awls and zigzags (Fig. 1). The first two types make up about 15% of the total number of hairs, and are collectively called overhairs. The zigzags make up over 80% of the total, and constitute the underfur. The guard hairs and the zigzags are almost circular in cross-section, while the awls are bean-shaped. The guard hairs have no constriction along their length, the awls may have one, and the zigzags have three or four. The whole coat is periodically renewed by moulting.

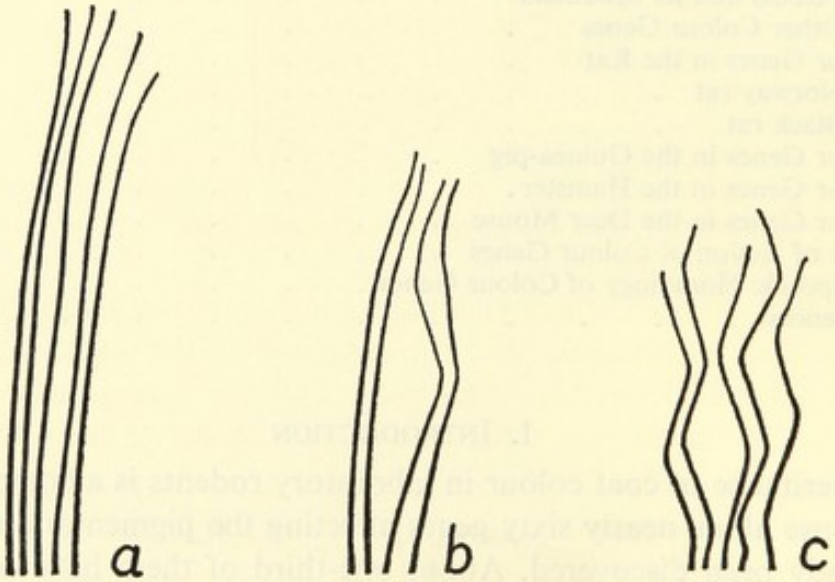

Fig. 1. The three types of hairs: (*a*) guard hairs; (*b*) awls; (*c*) zigzags.

The hair is formed in the hair follicle, which is a deep epidermal pouch sunk into the dermis (Fig. 2). The part above the follicle is a dead structure composed of keratinized cells compactly cemented together. In the cross-section of the hair three layers can be distinguished. These are, starting with the outermost, cuticle, cortex and medulla (Fig. 3). The cuticle consists of translucent scales without any pigment. The cortex consists of elongated cells with only a small amount of pigment. The mass of the hair is formed by the medulla, which is composed of loosely arranged cells of irregular shape, heavily loaded with pigment in dark animals.

The pigment found in rodent hairs is melanin. It occurs in the form of granules within the cells and not between them. It is formed by specialized

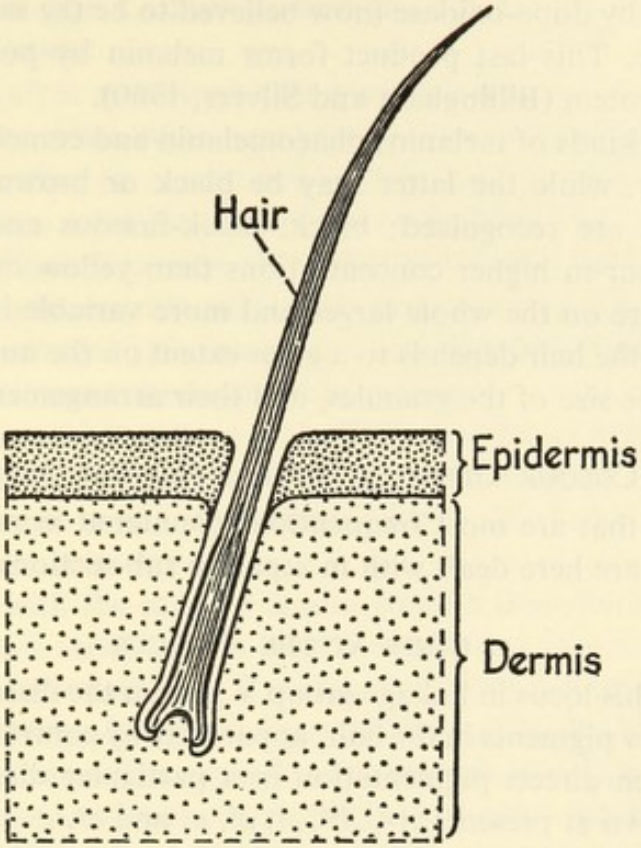

FIG. 2. Diagram of a hair follicle.

cells called melanocytes, which originally come from the neural crest—except those in the eyes, which come from the outer wall of the optic cup. Melanin is a complex polymer derived from tyrosine. This colourless amino acid is first hydroxylated by the enzyme tyrosinase to a substance called dopa, and then

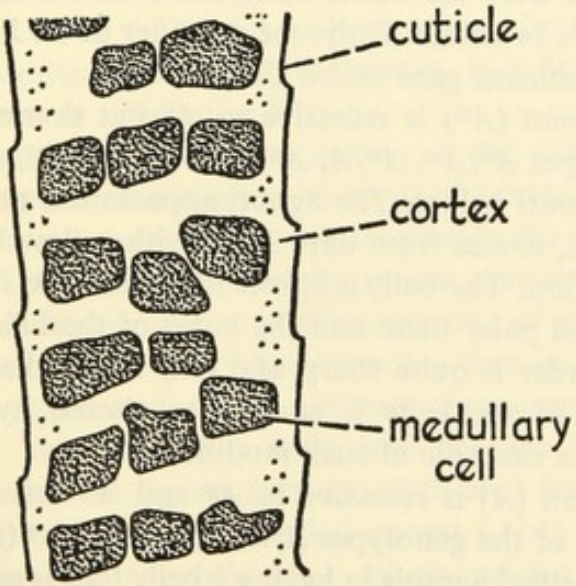

FIG. 3. Longitudinal section of a hair.

dopa is oxidized by dopa-oxidase (now believed to be the same as tyrosinase) to dopa-quinone. This last product forms melanin by polymerization and coupling with protein (Billingham and Silvers, 1960).

There are two kinds of melanin: phaeomelanin and eumelanin. The former is always yellow, while the latter may be black or brown. Three different shades of black are recognized: black, black-fuscous and fuscous. Black pigment can occur in higher concentrations than yellow or brown, and the black granules are on the whole larger and more variable in shape and size.

The colour of the hair depends to a great extent on the number, the colour, the shape and the size of the granules, and their arrangement within the cell.

III. Colour Genes in the Mouse (*Mus musculus*)

Colour genes that are most frequently encountered in routine laboratory work with mice are here dealt with in separate sub-sections.

A. Genes at the *A* locus

The genes at this locus in linkage group V govern the distribution of black, brown and yellow pigments in the hair, apparently by controlling some trigger mechanism which directs pigmentation in a particular direction. There are six of them known at present: A^y, A^w, A, a^t, a, and a^e.

Yellow (A^y) is the highest allele in order of dominance. It is lethal in the homozygous condition, A^y/A^y embryos dying at a very early stage. Mice of the genotypes A^y/A^w, A^y/A, A^y/a^t, A^y/a and A^y/a^e have a rich yellow or orange fur and black eyes. There is no eumelanin in the hairs, only phaeomelanin. In addition, they tend to grow very fat. In the presence of certain modifying genes, however, yellow mice may develop some black pigment, mainly in the cortical cells. The dark hairs are usually found along the middle of the back, but may also occur over the whole back and even the sides. Such mice are called sooty yellows or sables. Only one modifier of A^y has been isolated so far. It is a semi-dominant gene called *Umbrous* (U).

White-bellied agouti (A^w) is recessive to A^y but dominant over the rest. Mice of the genotypes A^w/A^w, A^w/A, A^w/a^t, A^w/a and A^w/a^e have dark agouti backs and light agouti bellies. The agouti appearance, named after a South American mammal, comes from dark hairs with yellow bands in the apical or sub-apical position. The belly is lighter than the back, because these bands are much wider and paler there and the bases of the hairs are not so dark. The back-belly border is quite sharp and may be marked by a few orange hairs. The colour of the belly is sometimes altered by modifying genes. "Snowy belly" is an example of such modification.

Grey-bellied agouti (A) is recessive to A^y and A^w but dominant over the other alleles. Mice of the genotypes A/A, A/a and A/a^e (for A/a^t see below) differ from white-bellied agoutis in having a belly that is only a little less dark than the back and no sharp border between the two. This form is often

referred to as "wild type", although the white-bellied agoutis have an equally good claim to this title.

Black-and-tan (a^t) is recessive to the three foregoing alleles and dominant over all the others. Mice of the genotypes a^t/a^t, a^t/a and a^t/a^e have glossy black backs and yellow or orange bellies, with a sharp border between them. The dorsal hairs are solid black, and the ventral hairs are yellow or orange with grey bases. There is usually a darker patch under the neck.

Non-agouti (a) is recessive to all the alleles except a^e. Mice of the genotypes a/a and a/a^e are entirely black except for a few light hairs behind the ears and some orange hairs round the nipples and genitals.

Extreme non-agouti (a^e) is the lowest allele in order of dominance. In a^e/a^e mice even the few light or orange hairs of the non-agouti form have disappeared. There is no trace of phaeomelanin in their fur (Hollander and Gowen, 1956).

In connection with the allele A it was implied that the usual dominance relationships of this series do not strictly apply to A and a^t. A/a^t mice have agouti backs and light bellies, as if A were dominant over a^t for the back and a^t over A for the belly. There has been much speculation about the cause of this interesting phenomenon, and for a thorough discussion of the subject the reader is referred to Grüneberg (1952). A simpler explanation has been given recently by Hollander and Gowen (1956) who suggest that the production of less black may be regarded as dominant over the production of more black throughout the series.

B. GENES AT THE *B* LOCUS

The four alleles at this locus in linkage group VIII determine the type of eumelanin (black or brown) that will be produced. They are B, b, b^c and B^{lt}.

In describing the effects of the genes of the agouti series in the last subsection it was assumed that the mice were homozygous for the allele *Black* (B), which causes the production of black eumelanin. It is dominant over b and b^c and semi-dominant in relation to B^{lt}.

The allele *brown* (b), which is recessive to all the others, causes brown eumelanin to be produced instead of black in the homozygous condition. This happens wherever eumelanin is present, even in the eyes. The colour of phaeomelanin remains unaffected. As the brown granules are smaller than the black ones, and the maximum possible number of granules in a cell is limited, the amount of melanin in b/b mice is less than in B/B. When in mice of the agouti series B/B is replaced by b/b their phenotypes are altered in the following manner: yellow ($A^y/-$; B/B) mice become brown-eyed yellow ($A^y/-$; b/b), white-bellied agouti (A^w/A^w; B/B, etc.) become white-bellied cinnamon (A^w/A^w; b/b, etc.), grey-bellied agouti (A/A; B/B, etc.) become dark-bellied cinnamon (A/A; b/b, etc.), black-and-tan (a^t/a^t; B/B, etc.) become chocolate-and-tan (a^t/a^t; b/b, etc.) and non-agouti (a/a; B/B) become

chocolate (*a*/*a*; *b*/*b*). In addition to its effects on eumelanin, *b* may slightly reduce body size.

The allele *cordovan* (b^c) is dominant over *b* but recessive to *B* and B^{lt}, and resembles *b* in its effects except that the fur of b^c/b^c and b^c/b mice is deeper and richer in tone like cordovan leather.

The semi-dominant allele *Light* (B^{lt}) not only affects the pigment qualitatively but also quantitatively. B^{lt}/B^{lt} mice have almost white hairs with brownish tips. In $B^{lt}/-$ mice, known as dark, the hairs are very light grey with darker tips. When this gene is present in agouti mice the difference between B^{lt}/B^{lt} and $B^{lt}/-$ animals becomes less conspicuous, because the yellow band encroaches on the coloured tip.

C. GENES AT THE *C* LOCUS

The five alleles at this locus in linkage group I determine the amount of pigment, phaeomelanin as well as eumelanin, that will be produced. They are *C*, c^{ch}, c^e, c^h and *c*.

In describing the effects of the genes at the *A* and *B* loci it was assumed that the mice were either homozygous or heterozygous for the allele *Full colour* (*C*), which is necessary for the full production of pigment. *C* is completely dominant over the other alleles (for exceptions see below and subsection E).

The allele *chinchilla* (c^{ch}) reduces the number of phaeomelanin granules per cell, and so decreases the intensity of yellow considerably. The number of eumelanin granules is not appreciably affected, but their size is reduced, and this results in a change of shape from oval to round and a change of colour from intense black to fuscous. In the homozygous condition it changes the colour of agouti mice from brownish grey to a purer grey resembling that of a chinchilla—hence the name. It also makes yellow mice ivory ($A^y/-$; c^{ch}/c^{ch}) and non-agouti mice dull black or sepia (*a*/*a*; c^{ch}/c^{ch}). Black-and-tan mice with c^{ch}/c^{ch} have a dull black back and a nearly white belly. This allele has practically no effect on chocolate (*a*/*a*; *b*/*b*) mice, probably because the brown granules are already rather small and round, and so outside the range of its effects. The eyes of c^{ch}/c^{ch} mice are somewhat less densely pigmented, especially during the first month.

Extreme dilution (c^e) suppresses the formation of phaeomelanin completely and reduces the amount of eumelanin considerably, with the result that yellow mice homozygous for c^e become pure white, agouti mice dirty white and non-agouti mice pale brown. The dilution of the brown pigment in cinnamon and chocolate mice is a little less marked. The eyes of c^e/c^e mice are dark, and the baby-coat is even lighter than the adult one.

The allele *himalayan* (c^h) in the homozygous condition causes a phenotype similar to a Himalayan rabbit. The baby coat of c^h/c^h mice is pale tan, but after the first moult the nose, ears, tail and scrotum become darker while the

rest of the body becomes lighter. The feet never darken, and the eyes, although unpigmented at birth, become ruby by weaning time. The effects of c^h are more intense than those of c^e if we go by the colour of the body, and less intense if we go by the colour of the extremities (Green, 1961).

Albino (*c*) suppresses the formation of any pigment, phaeomelanin or eumelanin, completely. The fur of *c*/*c* mice is snow-white and the eyes pink, regardless of any other colour genes they may carry. Albino mice can be distinguished from their coloured litter-mates as early as the 12th day of gestation, when the eye pigment begins to form in the retina.

As mentioned before, *C* is dominant over c^{ch}, c^e, c^h and *c*. The last four alleles form compounds with one another which are roughly intermediate between the two homozygotes concerned. For instance, the colour of c^{ch}/c^e mice falls about half-way between that of c^{ch}/c^{ch} and c^e/c^e. Leaving out c^h, which complicates matters by its localized action, we get a gradually decreasing intensity of colour in the following series: c^{ch}/c^{ch}, c^{ch}/c^e, c^{ch}/c, c^e/c^e, c^e/c and c/c.

The dominance of *C* over *c* does not appear to be always complete: *C*/*c* mice have been known to turn wholly or partially white in later life. Whether a change of dominance is involved is not clear, except when it consistently happens in the presence of another gene (see sub-section E). It has also been reported that *C*/*c* mice can be distinguished from *C*/*C* if they are depilated with the minimum dose of X-rays that is necessary: the regenerating hairs are mostly white in *C*/*c*; *A*/*A* mice but dark in *C*/*C*; *A*/*A* mice. However, Chase (1949) was unable to confirm this.

The phenotypical effects of these genes on the intensity of colour, and their microscopic effects on the number and size of pigment granules are paralleled by alterations in the activity of the enzyme dopa-oxidase (see section II) in the hair follicles. This strongly indicates that pigment formation is enzymatically controlled.

D. GENES AT THE *D* LOCUS

So far we have dealt with loci that determined either the type of pigment produced or its quantity. Now we come to the three alleles at the *D* locus in linkage group II that are concerned with the distribution of pigment granules in the hair cells. These are *D*, *d* and d^l.

In describing the effects of the genes at the *A*, *B* and *C* loci it was assumed that the mice were either homozygous or heterozygous for the allele *Intense* (*D*), which is necessary for the normal distribution of pigment granules in the hair cells, and which is dominant over the other two.

The allele *maltese dilution* (*d*) in the homozygous state leads to the clumping of pigment granules, which occur in irregular masses instead of being fairly uniformly distributed. In the eyes clumping is more pronounced in the retina than in the iris. About one-third to two-thirds of the pigment present is so

affected, regardless of its type. The total amount of pigment is slightly increased, and this is reflected by a slight increase in the activity of dopa-oxidase. Clumping of pigment granules results in a dilution of the fur colour. Mice of the black (*B*/*B*) agouti series when homozygous for *d* have the following phenotypes: $A^y/-$; *d*/*d* mice are dilute yellow with black eyes, A^w/A^w; *d*/*d* dilute agouti with white bellies, *A*/*A*; *d*/*d* dilute agouti, a^t/a^t; *d*/*d* blue-and-tan and *a*/*a*; *d*/*d* blue or blue-grey. When *B*/*B* is replaced by *b*/*b* in the above genotypes they become brown-eyed dilute yellow, white-bellied dilute cinnamon, dilute cinnamon, dilute chocolate-and-tan and dilute chocolate respectively. Dilute yellow and agouti mice, whether black or brown, have a streaky, washed-out, faded appearance. Dilute chocolate animals are the colour of milk chocolate. In addition to its effects on coat colour, *d* increases body size slightly.

Dilute-lethal (d^l) affects the fur in the same way as *d*, but d^l/d^l mice die at about three weeks.

There have been two or three reports of possibly +/*d* mice being dilute when young and intense later on (the normal allele will henceforward be written as +).

E. GENES AT THE *P* LOCUS

The four alleles at this locus in linkage group I govern not only the amount of pigment both, phaeomelanin and eumelanin, in the fur and eyes, but also the shape, size and distribution of individual granules. They are *P*, *p*, p^r and p^s. *P* is dominant over all the others, p^r over *p* and p^s, and *p* over p^s.

The genotypes considered in previous sub-sections were assumed to be homozygous or heterozygous for *Intense* (*P*), which is necessary for the normal production and distribution of pigment.

Pink-eye (*p*) in the homozygous state reduces the amount of eumelanin considerably and phaeomelanin slightly. Eumelanin granules are fewer, especially in the cortical cells, and lack sharp outlines. They are irregular in shape, somewhat filamentous, and tend to form large flocculent clumps. Phaeomelanin granules are of normal shape and size, only their number is reduced. The gene is named after its effects on the eyes, which in *p*/*p* mice are pink, resembling those of albinos, although they are not so completely devoid of pigment. Agouti mice (*B*/*B* or *b*/*b*) homozygous for *p* have an orange or yellow fur, similar to that of $A^y/-$ mice, except that the hairs have dull or slate-grey bases. The colour of non-agouti pink-eyed mice (*a*/*a*; *B*/*B*; *p*/*p*) is described as "blue lilac", which is a lighter version of maltese blue (*a*/*a*; *B*/*B*; *d*/*d*). The colour of chocolate pink-eyed mice is described as "champagne" or "café-au-lait", which is a lighter version of dilute chocolate (*a*/*a*; *b*/*b*; *d*/*d*). Pink-eyed yellow ($A^y/-$; *p*/*p*) mice are practically the same colour as plain yellow mice. As c^{ch} greatly reduces phaeomelanin and *p* eumelanin, chinchilla pink-eyed (c^{ch}/c^{ch}; *p*/*p*) mice have cream fur. The genotype c^e/c^e; *p*/*p* is

completely white. Pink-eyed mice are also slightly smaller than their normal litter-mates.

The allele *Japanese ruby* (p^r) has a weaker effect on the pigment of the eyes and fur. The eyes of p^r/p^r mice vary from pink to black, and may not be of the same colour. The fur resembles that of p/p mice, except that it is less diluted and the amount of phaeomelanin is normal. The colour of a/a; B/B; p^r/p^r mice is about half-way between that of a/a; B/B; p/p (blue lilac) and a/a; B/B; $+/+$ (non-agouti black) mice.

The allele *sterile* (p^s) affects coat colour in the same way as p, but in addition p^s/p^s mice are retarded, and have abnormal spermatogenesis and uncoordinated movements (Hollander *et al.*, 1960).

Pink-eye also modifies the dominance of C over c. The colour of a/a; B/B; p/p and a/a; b/b; p/p mice is lighter when they are C/c than when they are C/C. Similarly, the dominance of B over b is incomplete in certain genetic milieu if p is present.

F. *Piebald* AND ITS MODIFIERS

Piebald (s), a recessive gene in linkage group III, produces a type of spotting that falls between the regular and symmetrical pattern of hooded rats and the erratic distribution of white and pigmented areas seen in spotted guinea-pigs. White and coloured areas in s/s mice are clearly defined, the colour of the latter depending on the genes at other loci. The degree of spotting is highly variable: s/s mice range from almost self-coloured with very few spots to entirely white forms, called "all white" to distinguish them from albinos (c/c). There is as a rule more white on the belly than on the back, and the commonest centres of depigmentation are the feet, tail (especially the tip), nose, and small areas round the umbilicus and between the eyes. Shoulder spots may join to form a collar, and lumbar spots may similarly form a belt. When the degree of spotting is increased by selection, the last regions to turn white are the ears, eyes, cheeks and haunches. The eyes are dark even in "all white" mice. However, the eye pigment is found only in the retina, and not in the iris and choroid as well. There is no dopa-oxidase activity in the white areas.

The extent of the white area in s/s mice is determined by a number of modifiers, called "k" genes, with cumulative effects. The modifiers may be regarded as spotting genes in their own right, for they sometimes produce spotting in $+/s$ and even $+/+$ mice. When this occurs, $+/s$ mice are found to have a larger white area than $+/+$, which means that the "k" genes also act as modifiers of the dominance of the normal allele ($+$) over s. Yet another peculiarity of the "k" genes is that although they are recessive in the absence of s, in s/s mice they become semi-dominant, so that animals heterozygous for the "k" genes are more spotted than those without them. This means that s in turn acts as a dominance modifier of the "k" complex. It must by now be

clear to the reader that piebald spotting is best treated as a quantitative character.

G. OTHER COLOUR GENES

The remaining colour genes, less frequently encountered in routine laboratory work with mice, are dealt with together in this sub-section, regardless of whether they affect coat colour severely or mildly. They are arranged in alphabetical order, according to their symbols.

Belted (*bt*, VI), a recessive gene, produces white spotting of a more regular pattern than *s*. In *bt*/*bt* mice there is a white belt just behind the middle of the back, which may or may not be complete ventrally. Occasionally, +/*bt* mice have spots on the belly.

Black-eyed-white (*bw*) in homozygous condition produces pure white fur and black eyes. However, a few pigmented hairs may occur dorsally. Mice of the genotype *bw*/*bw*; *p*/*p* resemble albinos (*c*/*c*), for *p* makes the eyes pink (Kreitner, 1957).

Flexed-tail (*f*) is a recessive gene affecting the blood and skeleton, but *f*/*f* mice usually have white spots on the feet, tail and belly in addition.

Grizzle-belly (*Gb*) is a dominant gene, its name being an apt description of its effects (Schaible, 1960).

The recessive gene *grey-lethal* (*gl*), besides seriously affecting the skeleton, suppresses the formation of phaeomelanin either completely or partially, with the result that *A*/*A*; *B*/*B*; *gl*/*gl* mice are slate-grey and *A*/*A*; *b*/*b*; *gl*/*gl* mice a dilute shade of cinnamon. The gene has no noticeable effect on the coat of *a*/*a* and c^e/c^e mice, where there is no phaeomelanin present anyway.

Leaden (*ln*, XIII) is a mimic of *d* (see sub-section D), but chocolate leaden (*a*/*a*; *b*/*b*; *ln*/*ln*) animals are a little lighter than chocolate dilute (*a*/*a*; *b*/*b*; *d*/*d*). Chocolate mice homozygous for both *d* and *ln* are like plain chocolate leaden mice. The clumping of pigment granules produced by *ln* is a little severer than that produced by *d*.

Misty (*m*, VIII) dilutes the colour of the fur slightly. Non-agouti and chocolate mice are darker when *m*/*m* than when *ln*/*ln* or *d*/*d*. Misty mice have white tail-tips and occasionally also a spot on the belly.

Mahogany (*mg*, V) in homozygous condition makes agouti fur much darker, *A*/*A*; *mg*/*mg* mice looking like umbrous agoutis. Darkening is strongest down the middle of the back, where the yellow bands of the hairs are much reduced, and weakens towards the sides. Ventral hairs are solid grey with no yellow bands. The tail and the ears are also darkened (Lane and Green, 1960).

Microphthalmia (*mi*, XI), a semi-dominant gene, sometimes leads to spotting in the heterozygous condition. The homozygotes are always completely white with small pink eyes, and in addition have gross abnormalities of the skeleton. Another semi-dominant gene at this locus is *White* (Mi^{wh}), which

produces a lighter version of blue (*a*/*a*; *B*/*B*; *d*/*d*) in the heterozygous condition. $M_i^{wh}/+$ mice also have white spots on the feet, tail-tip and belly, and sometimes small ones on the back, consisting of just a few white hairs. The eyes of $M_i^{wh}/+$ mice are dark ruby if they are $B/-$; $D/-$, and red if they are $B/-$; *d*/*d*. The coat of the latter genotype is pastel grey. Mi^{wh}/Mi^{wh} mice with their snow-white fur and pink eyes resemble albinos, except that their eyes are smaller and their fertility below normal.

Mottled (*Mo*, XX), a sex-linked semi-dominant gene, leads to a phenotype characterized by light, usually off-white, hairs scattered all over the body. The shade of off-white is darker in black mice than in brown. Hemizygous males ($Mo/-$) die before birth, and all affected females are $Mo/+$. Another gene at this locus, *Brindled* (Mo^{br}), resembles Mottled in every respect, except that $Mo^{br}/-$ males die when they are about two weeks old. Yet another allele of *Mo*, named *Dappled* (Mo^{dp}), has been described recently (Phillips, 1961). $Mo^{dp}/+$ females have a coat resembling that of $Mo/+$, but differ from them in several other respects. It is probable that the mutant genes reported by Lyon (1960) and Welshons and Russell (1959) were recurrences of one of these alleles.

The gene *pallid* (*pa*, V) resembles *p* in its effects, but the coat of *pa*/*pa* mice is lighter than that of *p*/*p*, and double homozygotes (*pa*/*pa*; *p*/*p*) are lighter than the single ones (*pa*/*pa* or *p*/*p*). Pallid seems to dilute all three types of pigment. Yellow ($A^y/-$) mice with *pa*/*pa* are light lemon in colour. In addition to its effects on coat colour, *pa* slightly reduces body size and causes abnormalities of the labyrinth.

Pearl (*pe*) also reduces the amount of all three pigments. In agouti pearl (*A*/*A*; *pe*/*pe*) mice the whole hair is light, the base more so than the tip. Occasionally a long, narrow, white streak may be seen along the midline on the ventral side of both *pe*/*pe* and $+/pe$ animals. Non-agouti pearl mice look like non-agouti ruby (see below), but may be distinguished by the lighter bases of the hairs. The colour of *a*/*a*; *b*/*b*; *pe*/*pe* (chocolate pearl) mice is about the same as that of *a*/*a*; *b*/*b*; c^{ch}/c. The effects of *pe* and *b* on the eyes are additive: adult *a*/*a*; *b*/*b*; *pe*/*pe* mice have ruby eyes. Yellow pearl ($A^y/-$; *pe*/*pe*) mice are cream coloured.

Patch (*Ph*, III), a semi-dominant gene, resembles *s* and *bt* in its effects. $Ph/+$ mice have sharply defined white patches, with no intermixture of white and pigmented hairs. The extent of the white area on the back is highly variable, but there is always a large spot on the belly, together with some on the tail and feet. There are no spots on the head. The homozygotes die before birth (Grüneberg and Truslove, 1960).

Ruby-eye (*ru*, XII) slightly reduces both eumelanin and phaeomelanin. Non-agouti ruby-eyed (*a*/*a*; *B*/*B*; *ru*/*ru*) mice are a dull dark sepia or dark slate in colour. The eyes of *ru*/*ru* animals have no pigment at birth, but it starts developing during the first week. In general *ru*/*ru* mice are like p^r/p^r

mice, even to the extent of having asymmetrically affected eyes varying from pink to ruby.

Silver (*si*, IV) in the homozygous condition produces a phenotype characterized by the presence of hairs which may be white; black; black with white tip; or black or grey with several white bands. There are two types of *si*/*si* mice: light silvers, in which white hairs predominate, and dark silvers, in which black hairs predominate. The males are usually more strongly affected than the females. Silvering becomes more intense with age in non-agouti mice, but weakens in agouti and yellow mice. The gene is not always recessive: some +/*si* mice have a few white hairs, usually on the belly, where silvering is most marked in *si*/*si* mice. Its effects in chocolate mice are less intense than in non-agouti mice. Silver agouti and silver yellow mice have a more or less white underfur. It seems that the gene *b* in heterozygous condition acts as a modifier of *si*: *a*/*a*; *B*/*b*; *si*/*si* and a^t/a^t; *B*/*b*; *si*/*si* are lighter than they would be if they were either *B*/*B* or *b*/*b*, and nearly the whole of their underfur is white.

Steel (*Sl*), a semi-dominant gene, lethal in the homozygous condition, causes a dilution of the fur in agouti mice, which is most marked on the belly (Sarvella and Russell, 1956). The ears, feet and tail in *Sl*/+ mice are light, and the tip of the snout is white. There is also a white spot on the forehead and the belly, and occasionally a few white hairs on the back. In addition, *Sl*/+ mice are anaemic. The interaction of *Sl* and W^v will be discussed later.

Splotch (*Sp*, XIII) is a semi-dominant gene, lethal in the homozygous condition. Heterozygotes have white spots on the belly, and occasionally also on the back. The feet are usually white.

Tabby (*Ta*, XX) is a sex-linked semi-dominant gene. *Ta*/+ females have transverse black stripes like the tabby markings of cats. In *Ta*/*Ta* females and *Ta*/− males the skin and hair structure are also affected.

The recessive gene *light head* (*te*, III), besides being a modifier of *s*, is a spotting gene in its own right. In *te*/*te* mice there are a few white hairs on the forehead, while in *te*/*te*; *s*/*s* mice the whole head is white.

Tortoise-shell (*To*, XX), another sex-linked semi-dominant gene, produces a phenotype resembling a tortoise-shell cat, the main difference being that there is less yellow in the mouse. *To*/+; *s*/*s* mice retain the characteristic features of both *To*/+ and *s*/*s* animals. *To*/*To* females and *To*/− males do not survive. This gene also affects hair structure and the skeleton.

Taupe (*tp*, I) resembles *ru* in its effects, the chief differences between *tp*/*tp* and *ru*/*ru* mice being that in the former the eyes are not affected, and the belly fur is lighter with traces of yellow at the margins.

Varitint-waddler (*Va*, XVI), a semi-dominant gene, in the heterozygous condition produces a peculiar mixture of spotting and dilution. Islands of fully coloured fur occur, but do not cross the midline either dorsally or ventrally. The dilute areas become almost white in old *Va*/+ mice, while

some patches, commoner on the underside, are white from the beginning. *Va*/*Va* mice are completely white, except for small fully coloured spots near the ears and the base of the tail. This gene also affects the labyrinth, and, consequently, behaviour.

Dominant spotting (*W*, III) acts as a semi-dominant gene in most laboratory stocks (see below), *W*/+ mice having white spots. The spots on the belly have clearly defined edges, but those on the back do not. In addition, the back is variegated, that is the white and coloured areas are broken up and thoroughly intermingled, with white hairs occurring in pigmented regions. On the whole, there is more white per unit area on the belly than the back. The degree of spotting is almost entirely dependent on modifying genes of the m(*W*) complex, which have little or no effect in the absence of *W*. These genes are probably three in number, all recessive. *W*/+ mice homozygous for all or most of them are about 90% white, whereas the same mice homozygous for the normal alleles of the m(*W*) complex would be almost self-coloured, with an occasional small belly-spot. This means that the genes of the m(*W*) complex act as modifiers of the dominance of *W* over its normal allele (+). Since most laboratory stocks carry some of these genes, *W* commonly acts as a semi-dominant factor. *W*/*W* mice die of anaemia soon after birth, but if they live long enough to grow fur they resemble black-eyed whites, regardless of the m(*W*) complex. *W*/+ ; *s*/*s* mice are white, with an occasional small pigmented area near the ears or on the haunches, and have black eyes. The gene A^y appears to act as a modifier of *W*: there is less white in A^y/− ; *W*/+ mice than in +/+ ; *W*/+. Another semi-dominant gene at this locus is W^v, which reduces the number of both eumelanin and phaeomelanin granules and causes a general dilution of the fur. Sometimes granule size is also affected. W^v/W^v mice are white with black eyes, and suffer from anaemia and sterility. W^v/+ ; *Sl*/+ mice are completely white ventrally and heavily mottled dorsally, with the remaining pigmented hairs on the back lighter than in either W^v/+ or *Sl*/+ mice. Yet another allele of *W* is W^j. W^j/+ mice have more ventral white than *W*/+, and also some spots on the crown of the head. W^j/W^j mice are white with black eyes, and die of anaemia after about two weeks (Russell *et al.*, 1957).

IV. Colour Genes in the Rat (Genus *Rattus*)

The two types of rats usually kept in laboratories are the Norway rat (*R. norvegicus*, sometimes called *Mus norvegicus*) and the black rat (*R. rattus*). As they differ in several respects with regard to the inheritance of coat colour, it is advisable to deal with them separately.

A. NORWAY RAT

In contrast with the mouse, there are only two genes at the agouti locus in the Norway rat: *A* and *a*. They resemble the corresponding genes in the

mouse in their effects: A/A and A/a animals are agouti with grey bellies, and a/a animals are non-agouti (black).

The effects of the two genes at the *B* locus, *B* and *b*, are also similar to those of the corresponding genes in the mouse. Agouti rats become cinnamon with b/b, and non-agouti rats chocolate.

The genes *C* and c^a at the albino locus correspond to *C* and *c* in the mouse respectively: c^a/c^a rats have no pigment in the fur or eyes. It is possible that another allele, ruby-eyed (c^r), with effects similar to those of c^{ch} in the mouse, also exists.

The gene *d* also occurs in the Norway rat. The coat of d/d animals is light yellowish or reddish grey.

Another gene for dilution is *fawn* (*f*). Its effects are not as marked as those of *d*, but supplement the latter when an animal is homozygous for both genes. Non-agouti rats with f/f are black fawn, and with f/f; d/d blue fawn. The latter colour is lighter than the blue of d/d rats.

The *H* locus may be regarded as partly corresponding to *S* in the mouse, but only partly, for the mouse has no real counterpart of the regular pattern of the hooded rat. The normal allele *Intense* (*H*) is dominant over all the others. Next in order of dominance comes *Irish* (h^i). Rats of the genotype h^i/h^i have a white spot of variable size on the ventral side between and behind the front legs. The allele *hooded* (*h*) is recessive to *H* and h^i. In h/h rats the entire ventral surface posterior to the head is white, and dorsally pigment is restricted to the head and shoulders and a central stripe of variable width running to the tip of the tail. The stripe is sometimes broken, and may be absent altogether. The extent of the pigmented area is dependent on modifying genes. The allele *notched* (h^n) is recessive to *H*, but forms identifiable compounds with h^i and *h*. In h^n/h^n rats the extent of the white area is as great as in extreme cases of hooded, or still greater. The white area may even cover a part of the head dorsally. h^n/h^i rats have an entirely white belly and a few white hairs on the sides. The compound h^n/h resembles h^n/h^n more closely than h/h.

The gene *pink-eyed yellow* (*p*) is roughly similar to *p* in the mouse in its effects: p/p rats have bright yellow coats and pink eyes.

Red-eyed yellow (*r*) is a mimic of *p*.

The gene *silver* (*s*) produces an intermixture of white and coloured hairs. This does not occur in the juvenile coat, and s/s rats can be confidently distinguished only after 6 to 8 weeks. The coat becomes lighter with age, and finally all hairs become either completely white or white with black tips.

B. BLACK RAT

There are three genes at the agouti locus in the black rat: A^w, *A* and *a*. Their effects and dominance relationships are the same as in the mouse. The non-agouti black is sometimes called recessive black, to distinguish it from another type (see below) that also occurs in this animal.

The gene *b* is believed to occur in the black rat, because a wild cinnamon specimen was once observed.

The only two genes that occur at the albino locus are *Full colour* (*C*) and *albinism* (c^a), there being no alleles with intermediate effects.

The gene *d* has also been recorded; *d*/*d* rats are bluish grey or grey.

The genes at the *E* locus govern the spread of eumelanin in the fur. The normal allele *Extension* (*E*) is necessary for the genes of the agouti series to express themselves. Its recessive allele *e*, when homozygous, suppresses the spread of eumelanin to the fur, restricting it largely to the eyes and the skin of the ears and the tail. The fur of *e*/*e* rats is consequently cream or creamy white or ochraceous buff, the eyes dark and the skin of the ears and the tail slaty. It also has a dominant allele, *Super-extension* (E^D), which leads to the spread of the black pigment everywhere in the fur of $E^D/-$ rats, regardless of any genes of the agouti series they may carry. This type of black is called dominant black.

Grizzled, a recessive gene, produces a phenotype roughly resembling *si*/*si* mice. Its effects become apparent only after the age of 6 months. Grizzled rats have from 5 to 75% of their hairs white, and these are scattered all over the body.

V. Colour Genes in the Guinea-pig

Several species of the genus *Cavia* have been studied genetically. Coat colour is inherited in much the same manner in all of them. Separate treatment of each one is considered unnecessary.

The three genes at the agouti locus, A^w, *A* and *a*, have approximately the same effects as the corresponding genes in the mouse.

The gene *B* has mutated to *b* in the guinea-pig also. The presence of certain modifiers and the accumulation of the normal alleles at the *C*, *P* and *F* loci in *b*/*b* animals may result in a curious type of dilution called dinginess (Wright, 1959a).

There are five genes at the albino locus: *Intense* (*C*), *dark dilution* (c^k, also referred to as c^s), *light dilution* (c^d), *red-eyed dilution* (c^r) and *albinism* (c^a). Each one except *C* reduces both phaeomelanin and eumelanin a little more than the one that comes before it. *C* is dominant over the rest, which form intermediate compounds with one another. In the following list all possible genotypes are arranged in a descending order according to the intensity of colour: $C/-$, c^k/c^k, c^k/c^d, c^k/c^r, c^k/c^a, c^d/c^d, c^d/c^r, c^d/c^a, c^r/c^r, c^r/c^a, c^a/c^a. The eyes are black in the first eight genotypes, dark red in the ninth, light red in the tenth and pink in the eleventh. Even c^a/c^a guinea-pigs are not complete albinos like *c*/*c* mice: they have some pigment left in the extremities, and suggest the Himalayan pattern of rabbits. For this reason c^a is sometimes written as c^H.

Diminished (*dm*) controls the residual variability of the intensity of colour

in animals with moderate dilution due to the action of genes at the *C* locus (Wright, 1959b). It has no effect on *C*/− genotypes, except in combination with *si* (see below).

There are three genes at the *E* locus: *E*, e^p (also referred to as e^t) and *e*. The action of *E* and *e* is the same in the guinea-pig as in the black rat, and e^p (*partial extension*) in homozygous condition produces the tortoise-shell pattern. Occasionally *E*/− and e^p/e^p genotypes overlap. The compound e^p/e is very variable, ranging from being intermediate between e^p/e^p and *e*/*e* to being indistinguishable from the former. It may be mentioned here that yellow pigment in high concentration appears red in the guinea-pig.

Fading yellow (*f*), an incompletely recessive gene, reduces phaeomelanin, *f*/*f* animals resembling c^k/c^k at birth, but fading later.

Grizzled (*gr*), also an incompletely recessive gene, has no effect at birth, but a progressive whitening of the dorsal hairs occurs later (Wright, 1959a).

Pink-eyed (*p*) has much the same effects as the corresponding gene in the mouse, except that no dilution of phaeomelanin takes place in *p*/*p* guinea-pigs. It supplements the action of *f*, so that *E*/− ; *f*/*f*; *p*/*p* animals are pale brownish cream.

Roan (*Ro*), an incompletely dominant gene, produces an intermixture of white and pigmented hairs in black, yellow (or red) and chocolate animals. Its expression is very variable, and in the presence of a modifier *Ro*/+ and +/+ genotypes may overlap.

The gene for white spotting (*s*, also referred to as s^p) is usually recessive, but in the presence of certain modifying genes +/*s* guinea-pigs may have some white spots. Two modifiers affecting its action, and perhaps also its dominance relationship with its normal allele, have been isolated. They are *Pi*−1 and *Pi*−2, and they act only when both are present. They may be regarded as spotting genes in their own right, for they can produce some spots in the middle of the forehead even in +/+ animals. Guinea-pigs homozygous for *s* and also carrying these modifiers are white, except for some pigmented spots round the eyes and the ears.

Silvering (*si*), which is also incompletely recessive, roughly resembles the corresponding gene in the mouse in its effects, although it may cause a slight dilution of the unsilvered fur too. The appearance of *si*/*si* guinea-pigs is extremely variable, severely affected animals being almost pure white except on the top of the head. In combination with *dm* it lightens the coat even more: *si*/*si*; *dm*/*dm* animals are wholly white except for one or two pale spots on the cheeks.

Whitish (*W*), an incompletely dominant gene, modifies the colour of chocolate and black hairs. The dinginess of *b*/*b* animals mentioned earlier may be caused by this gene.

VI. Colour Genes in the Hamster (Genus, *Cricetus* or *Mesocricetus*)

The only gene present at the *A* locus seems to be A^w. The normal coat of a hamster is agouti or golden brown on the back and white with an agouti band on the belly (Whitney, 1958).

The gene *C* for full colour has two mutant alleles: c^d, (partial albinism) and c^a (albinism). c^d/c^d hamsters have no pigment during the first three weeks, but some pigment develops later in the skin of the ears, scrotum, etc. They have pink eyes, which may occasionally turn ruby later. The gene c^d interacts with *ru* and *s* (see below). c^a/c^a animals are completely white.

The three genes at the *E* locus, *E*, E^D and *e*, act as they do in the black rat. The colour of *e*/*e* hamsters ranges from cream to orange, depending on the modifiers. They seem to have normal pigment in the skin of the ears, the rim of the eyelids and the hip gland.

The gene *ruby-eyed* (*ru*) dilutes black pigment to blue, and yellow to fawn. In *ru*/*ru* hamsters the hairs are medium blue sub-apically, and become greyish towards the roots. The belly is paler than the back, and the eyes are ruby. The males are completely or partially sterile, and viability is reduced in both sexes. Hamsters homozygous for both *ru* and c^d are practically full albinos.

Piebald (*s*) causes white spotting in the homozygous condition. It differs from the genes of the same name in other animals in an important respect: the edges of the spots are not sharp. Its expression is variable: *s*/*s* hamsters range from almost normal with a little white on the nose and face to more than half white. They are also rather small and have visceral abnormalities.

Light underfur, with the basal part of the hair lighter than the rest, occurs sporadically in young animals. The condition appears to be familial, but its manner of inheritance is not clear. The fur becomes normal in later life (Robinson, 1960).

VII. Colour Genes in the Deer Mouse (Genus *Peromyscus*)

The wild type gene at the *A* locus is A^w. It has a recessive allele which produces a variable number of agouti hairs in the pectoral region of the belly, and so may be regarded as roughly equivalent to the gene *A* of other rodents. There may possibly be another allele that changes the colour to buff, but the evidence for it is not satisfactory.

The gene *b* occurs in *Peromyscus*, but A^w/A^w; *b*/*b* animals are yellowish brown, considerably lighter than cinnamon mice.

The albino locus has three genes, *C*, c^{ch} and c^a.

The gene *d* also occurs in the deer mouse.

The *Extension* locus has only two alleles: *E* and *e*.

A recessive gene called *ivory* causes whitish brown fur in the young and dull white in adults. Ivory animals have no yellow pigment in the agouti band and

no black pigment in the cortical cells. The medullary pigment occurs in the form of brownish masses. Little (1958) thinks this might be an allele of *p* (see below).

The recessive gene *orange-tan* (*ot*) seems to be peculiar to the deer mouse. Young *ot*/*ot* animals are light buff-tan in colour, and adults yellow-tan or orange-tan. The colour is brightest on the sides and head. The tone of orange becomes richer with age. The white underparts and preauricular patches and the bicoloured tail are not affected by *ot* (Egoscue and Day, 1958).

Pink-eyed dilution (*p*), formerly called pallid, roughly resembles the corresponding gene in the mouse in its effects.

Silvering (*si*) produces a phenotype similar to silver mice. It interacts with *b*, so that *si*/*si*; *b*/*b* deer mice are paler than either *si*/*si* or *b*/*b*.

There is also a dominant gene for silvering. When first described, it was called *Grizzled*. Its effects, however, do not appear before the age of 6 months.

Hereditary white spotting in the deer mouse appears to be of several kinds. Castle (1940) has listed a gene *s* that causes white spots on the belly, feet and tail. Feldman (1936) has mentioned two recessive genes that produce spotting when they occur together, and at least one dominant spotting gene. All three of them together may produce white animals with black eyes.

Deer mice homozygous for the recessive gene *whiteside* have unpigmented hairs on the belly and sides. There is also some white on the top of the head, and the skin of the ears is pale. The formation of the agouti band is delayed in the dorsal hairs.

VIII. Mode of Action of Colour Genes

Most of the work described in this section was done on the mouse, but there is no reason to believe that it does not apply to other rodents.

The first efforts made to understand the mode of action of colour genes consisted in qualitative and quantitative studies of the pigments in the hairs, and measurements of the intensity of dopa-oxidase activity in the hair follicles of various genotypes. The results of these investigations and the conclusions drawn have already been given in the appropriate places.

Another type of approach, gratifying in its conception and success, has been made in recent years by Silvers and his collaborators (for an excellent review of this work and a full bibliography see Billingham and Silvers, 1960). It consists essentially in transplanting histocompatible skin grafts from near-term or neonatal donors to newborn recipients of a different constitution with regard to a given colour gene. The melanoblasts (immature forms of melanocytes) of the host enter and become incorporated in the growing follicles of the graft, and the colour of the hairs formed by the follicles shows whether the action of the gene in question takes place within the melanocytes or through the follicular environment. If the hairs of the graft are of the same

colour as the host then the gene has acted within the melanocytes, but if they conform to the genotype of the donor then the gene has acted through the follicular environment.

When the genotype of the host is *a*/*a* and of the graft $A^y/-$, the host melanocytes in the graft produce yellow pigment characteristic of the graft, and not the black that is characteristic of the host. In other words, the genotype of the hair follicles determines the type of pigment (or pattern if A^w or *A* is involved) that the melanocytes will produce regardless of their own genotype. So much so that when melanoblasts from an $A^y/-$ host enter an a^t/a^t graft they produce black pigment if the graft comes from the back, and a mixture of a little black and a lot of yellow if the graft comes from the belly. It has also been shown that $A^y/-$ melanocytes secrete eumelanin when grown in the spleens of mice of different genotypes. Clearly there can be little doubt that the genes at the *A* locus act through the follicular environment. They are further believed to affect the polymerization of melanin.

The genes at the *B*, *C* and *P* loci seem to act within the melanocytes. When the graft and the host differ with regard to any of these loci, the migrant melanocytes in the graft always produce the pigment they would have done if they had not left the host. Their own genotype determines the pigment they will produce, regardless of the genotype of the follicles in which they finally come to rest. Further, the *B* locus is believed to be concerned with melanin polymerization, the *C* locus with melanin synthesis and the *P* locus with the protein structure of the granules.

The genes at the *D* and *Ln* loci, which closely resemble one another in their effects, also act within the melanocyte but with a difference. The clumping of pigment granules characteristic of *d*/*d* and *ln*/*ln* melanocytes appears to be the consequence of their abnormal shape: they have fewer and finer dendritic processes. When *d*/*d* and *ln*/*ln* melanoblasts are transplanted into the anterior chamber of the eye of a mouse that is normal with regard to these loci, they grow into melanocytes which range from typically normal to typically abnormal with many intermediate grades. This shows that although the genes *d* and *ln* act within the melanoblast, their action may be modified by the environment of the cell.

The action of the spotting genes is less well understood. Spotting (it should be remembered that black-eyed white animals have just one large spot) differs from albinism in one important respect: the albino follicles contain clear cells, which are melanocytes incapable of secreting melanin on account of some biochemical block, but the follicles in white spots lack these cells. Billingham and Silvers (1960) suggest that these genes may cause spots either by interfering with the differentiation of the melanoblasts in the neural crest, or by interfering with the migration of the melanoblasts to all parts of the skin or by making the environment in certain areas hostile to the melanoblast.

IX. Interspecific Homology of Colour Genes

Strictly speaking, the homology of genes in different species can only be proved by crosses between them. Such crosses are usually impossible. In their absence circumstantial evidence may be taken into consideration. When genes in different species have similar effects, act in the same manner, give rise to similar new alleles by mutation, and have similar linkage relationships with other genes, they may be regarded as homologous. A complete correspondence with regard to all these points is not necessary; nor is it to be expected, considering that some species are more thoroughly investigated than others, and the genetic backgrounds of the genes always influence their expression.

There seems to be enough circumstantial evidence to allow one to speak of the interspecific homology of colour genes in laboratory rodents. The best examples are the genes at the *A*, *B*, *C*, *D*, *P* and *S* loci. More alleles are known at these loci in the mouse because it has been more intensively investigated than other animals. The significant fact is that when new mutations occur at these loci they follow the same pattern in more than one species.

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Chapter 7

Genetics of the Mouse

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I. INTRODUCTION

This chapter deals with the uses, sources, preparation and maintenance of special genetic stocks of mice, including mutant stocks, and those with genetic peculiarities such as chromosome translocations or particular combinations of histocompatibility genes.

It is aimed not at the professional geneticist, to whom the material in it will be already highly familiar, but at those who wish to use, or breed for use, these special genetic stocks while having no detailed knowledge of genetics or genetical techniques. It does, however, assume an understanding of simple genetic theory and terminology.

II. USES OF SPECIAL GENETIC STOCKS

A. MUTANT STOCKS

1. Ready-made deficiencies

For experiments requiring animals lacking some organ, hormone, or enzyme it may be possible to use mutants in which the deficiency is already present. Table I gives a list of such mutants, which is not intended to be exhaustive but merely to indicate the range of examples. Some of these mutants are only of potential interest, others have actually been put to use. One of the earliest uses of mutant mice was that of Keeler (1927). He used mice genetically blinded by the mutant *rodless retina* (*r*) to show that in path-finding behaviour sight was of much less importance than the tactile sense of the vibrissae. Dwarf mice, homozygous for the gene *dw*, which lack anterior

pituitary growth and thyrotrophic hormones (Ortman, 1956), have been used by Fønss-Bech (1947) for the assay of growth hormone, and by Bickis *et al.* (1956) and Bielschowsky and Bielschowsky (1959, 1960) to study the role of pituitary hormones in carcinogenesis. Genetically hairless mice (*hrhr*) have proved valuable in studying the effect of radiations on the skin (Crook *et al.*, 1958; Winkelmann, 1960), and mice genetically lacking bone resorption have been used in studying the metabolism of strontium (Carr, personal communication). These mice were homozygous for the gene *microphthalmia*, *mi*.

TABLE I

Examples of ready-made deficiencies resulting from mutant genes of the mouse

Deficiency	Gene	Gene name	References
Hair	*fs*	Furless	Green (1954)
	hl	Hairloss	Hollander and Gowen (1959)
	hr	Hairless	Grüneberg (1952)
Spleen	*Dh*	Dominant hemimelia	Searle (1959)
Pigment and tyrosinase	*c*	Albino	Grüneberg (1952); Foster (1951)
Hearing	*dn*	Deafness	Deol and Kocher (1958)
	vdf	Deaf	Kocher (1960)
Sight	*rd*	Retinal degeneration	Tansley (1951)
	r	Rodless retina	Grüneberg (1952)
Vestibular sense	*dr*, *je*, *kr*, *pi*, etc.	Dreher, jerker kreisler, pirouette, etc.	Grüneberg (1956)
Growth hormone	*dw*	Dwarf	Grüneberg (1952)
Glucuronidase activity	*g*	Low glucuronidase	Paigen and Noell (1961)
Bone resorption	*gl*	Grey-lethal	Grüneberg (1952)
	mi	Microphthalmia	Grüneberg (1952)

2. Resemblance to human diseases

Mutant mice with diseases resembling human ones may be used to make studies of the disease and carry out experiments which could not be done with human patients. The classical example of a mouse disease studied on account of its resemblance to a human one is, of course, cancer, and the various inbred strains which have been developed with genetically determined susceptibility or resistance to various types of tumour are in fact special genetic stocks within the meaning of this chapter. However, so much has already been written about mouse cancer, that mention here will be confined to other genetic diseases of the mouse. Examples of mutant genes of this type are given in Table II.

The two genes that have been most extensively studied in this connection are *dystrophia muscularis*, *dy*, and *obese*, *ob*. The great interest of *obese* lies in its resemblance to obesity and diabetes in man. The mutant homozygotes can be recognized at 4-6 weeks by their obesity; they are sterile and become highly hyperglycaemic by 9-10 weeks (Ingalls *et al.*, 1950; Mayer *et al.*, 1953).

The disease thus resembles the obese type of human diabetes. Much work has been done to find, on the one hand, the exact metabolic error and, on the other, ways of ameliorating the disease. It has been possible to make biochemical and pathological studies (e.g., on utilization of labelled acetate, or

TABLE II

Examples of mouse mutant genes producing diseases resembling human ones

Human disease	Gene	Gene name	References
Obesity and diabetes	*ad*	Adipose	Falconer and Isaacson (1959)
	ob	Obese	Ingalls, Dickie and Snell (1950)
Muscular dystrophy	*dy*	Dystrophia muscularis	Michelson, Russell and Harman (1955)
Various types of anaemia	*an, ja, Sl, W*-series, etc.	Anaemia, jaundiced, steel, dominant spotting, etc.	Grüneberg (1952); Stevens, Mackensen and Bernstein (1959); Sarvella and Russell (1956); E. S. Russell, Lawson and Schabtach (1957)
Eye anomalies	Cat	Dominant cataract	Paget (1953)
	lr	Lens rupture	Grüneberg (1952)
Retinitis pigmentosa	*rd*	Retinal degeneration	Tansley (1951)
Congenital malformations	*bp*	Brachypodism	Landauer (1952)
	cl	Club-foot	Robins (1959)
	pc	Phocomelia	Gluecksohn-Waelsch, Hagedorn and Sisken (1956)
Harelip and cleft palate	*Tw*	Twirler	Lyon (1958)
Kidney anomalies	*lx, Sd, se*	Luxate, Danforth's short tail, short-ear	Grüneberg (1952)
Various types of paralysis	*ax, ji, Lc, wl*, etc.	Ataxia, jittery, lurcher, wabbler-lethal, etc.	Lyon (1955); Grüneberg (1952); Phillips (1960a); Dickie, Schneider and Harman (1952).
Ichthyosis	*ic*	Ichthyosis	Grüneberg (1952)
Phenylketonuria	d^l	Dilute-lethal	Searle (1952b)
"Slipped discs"	*Pt*	Pintail	Berry (1961)

on body composition) that would not have been possible with man and to try out treatments with hormones and drugs, some of which proved toxic, so that their trial in man would have been undesirable. The exact metabolic error is still not known but there is an excess of circulating insulin (Christophe *et al.*, 1959). The work has been reviewed by Mayer (1955, 1957) who described the increase in knowledge concerning obesity that had been derived from comparisons between genetically obese mice and their litter-mates made obese by environmental methods. Since then at least two more types of genetically determined obesity have been described in the mouse, so that further extension

of these comparisons is now possible. These are a second recessive gene for obesity named *adipose*, *ad* (Falconer and Isaacson, 1959; Batt and Harrison, 1960) and a type of obesity which occurs in the inbred strain NZO (New Zealand obese) (Bielschowsky and Bielschowsky, 1956). Adipose homozygotes weigh about twice as much as their normal litter-mates, like *obob* are usually sterile, and are hyperglycaemic with sugar first appearing in the urine at 7-10 weeks (Latyszewski, 1961). By contrast, animals of the NZO strain are usually fertile, and can be restored to normal weight and blood sugar values by doses of stilboestrol. The exact mode of inheritance of the obesity in this strain is not known. In addition to their value for physiological studies, the mere existence of these three different types of inherited diabetes in the mouse is of interest in relation to the genetics of human diabetes since, if the disease can depend on several recessive genes, then the same might also be true in man and this could account for difficulties in the study of its inheritance.

The gene *dystrophia muscularis*, *dy*, is of course studied for its resemblance to human muscular dystrophy. It is already known that there are several distinct types of human inherited muscular dystrophy, but the disease caused by *dy* is the first suitable one to be discovered in animals. The gene *dy* is an autosomal recessive, the homozygous affected animals being recognized at about 2 weeks by their dragging hind legs. Genetic studies have shown that the clinical effect remains fairly constant on widely different genetic backgrounds (Loosli *et al.*, 1961). Extensive biochemical and pathological studies (recently reviewed by E. S. Russell, 1961) have uncovered many metabolic errors, but the fundamental one is still obscure.

Other interesting mutants producing diseases resembling human ones include *retinal degeneration*, *rd*, *dilute-lethal*, d^l, and *pintail*, *Pt*. Retinal degeneration resembles retinitis pigmentosa in man and its value lies in the fact that its pathology may be studied in all stages, whereas eyes affected with human retinitis pigmentosa are rarely available for pathological study (Sorsby, 1957.) Animals homozygous for dilute-lethal have diluted coat pigment and also develop convulsions and paralysis and die when about 3 weeks old (Searle, 1952b). Coleman (1960) has recently shown that they have greatly reduced phenylalanine hydroxylase activity and therefore resemble human individuals suffering from phenylketonuria. Pintail heterozygotes have shortened and kinky tails, and also reduced intervertebral discs which undergo rapid degeneration with age (Berry, 1961). Berry suggests that similar genetic conditions in man might lead to predisposition to "slipped discs".

B. MARKER STOCKS

For experiments in which cells, skin, ovaries, etc., of one animal are to be injected or grafted into another it is valuable for the donor cells to be marked in some way that will distinguish them from host cells.

An important group of genes in this respect are those affecting coat colour. Skin grafted from one animal to another is readily recognized if the hairs growing on it are of a different colour from those of the host. Moreover, young born from transplanted ovaries may be distinguished from those derived from host ovaries if it can be arranged that they shall differ in coat colour. For grafting work inbred strains are almost always needed, since the donor and host must be antigenically compatible. Thus, in order to colour-mark grafts, animals of the same inbred strain but differing in coat colour are required. These are available partly as a result of mutation of colour genes in already established inbred strains, and partly through the deliberate breeding of inbred strains heterozygous for particular mutants. Study of the list of inbred strains in *Standardized Nomenclature for Inbred Strains of Mice* (see "Sources of Stocks" below) shows that most of the best known strains have colour variants. The albino strains A and BALB/c have non-albino versions, A/Fa-+*c*, A/Be-*CRe* and BALB/c-*CFu*; strain CBA has three colour variants, CBA-*a*, CBA-a^t and CBA-*p*; and similarly, strains C3H, C57BL and DBA can all be obtained in mutant colour forms, C3H/Ha−*p*, C57BL-a^t, C57BL-*b*, DBA-*D* and DBA-*p*. All of these would be suitable for colour-marking grafts. The section below on ovarian transplantation describes the use of strains 129 and CBA/Fa-a^t for this technique.

Cytological marking is a second important and distinct type of marking. If the chromosomes of injected cells are recognizably different from those of host cells, then it is possible to tell for each dividing cell whether it is of donor or host origin. This method was first used to show that foreign haemopoietic cells injected into a mouse which has received a lethal dose of radiation settle down and repopulate the host's bone marrow (Ford *et al.*, 1956), giving an animal known as a radiation chimaera. These chimaeras have since been much studied (Ford, 1960; Welling *et al.*, 1959). The chromosome marker used was a translocation known as T6. Animals heterozygous for this translocation have a single abnormally small chromosome, while the homozygotes have two such chromosomes. This chromosome marker is now available in a stock coisogenic with strain CBA (Inbred Strains List *2*, 1961. See "Sources of Stocks" below).

Other characteristics which have been used for distinguishing host and donor cells include antigenic type (Owen, 1958) haemoglobin solubility and electrophoresis pattern (Popp *et al.*, 1958; Popp and Cosgrove, 1959; Welling *et al.*, 1959); and red-cell size and shape (Searle, 1952a; E. S. Russell *et al.*, 1956). In contrast to the cytological markers these give information concerning non-dividing cells.

C. ISOGENIC STOCKS

In the last paragraph it was mentioned that inbred strains are usually necessary for grafting work. This is in order to obtain homozygosity of genes

concerned in histocompatibility and applies to all work involving transplantation of tissue from one animal to another. This includes not only skin grafting and ovarian grafting, but also the implantation of tumours, or of blood-forming tissue. The ability of tissue to grow in a foreign host is thought to depend on similarity between donor and host in antigens formed by genes at a large number of loci (Prehn and Main, 1958) and the only reliable way of ensuring that donor and host do resemble each other in this way is to take them from the same inbred strain, so that both are homozygous for the same genes. (The way in which inbreeding leads to this homozygosis is discussed in the next chapter.) Such animals are referred to as being *isogenic* or *syngeneic* (Gorer *et al.*, 1961) with each other, and stocks of them are known as isogenic stocks.

Another use for isogenic stocks lies in studying in detail the action of a particular gene. Consider for example the gene *dy* for muscular dystrophy. If a mouse homozygous for *dy* is compared with another mouse taken at random the two animals may differ in respect of many genes in addition to *dy*. Hence any difference, let us say a biochemical one, found between these two animals might well be due not to *dy* but to some other gene. However, *dy* arose by mutation in an inbred strain, 129. Before this all animals of strain 129 were isogenic with each other, and hence the dystrophics, when they arose, differed from the other animals of the strain only in respect of the gene *dy*. Strain 129-*dy* thus constitutes an isogenic stock of *dy*, and any biochemical differences between dystrophics and normals found within it will depend on the gene *dy* only. When, as frequently happens, a gene arises by mutation in a stock that is *not* already isogenic, then a suitable isogenic stock may be prepared by special breeding, and methods of doing this are described below. E. S. Russell and her colleagues have made use of such lines in studies of hereditary anaemias of the mouse and possible treatment of such anaemias by implanting blood-forming tissue from normal animals. (E. S. Russell and Lawson, 1959; Bernstein *et al.*, 1959.)

Of particular interest are the isogenic-resistant or IR lines prepared by Snell (1948, 1958). By suitable breeding he obtained inbred strains of mice isogenic with an already known strain but resistant to a transplantable tumour to which the strain was normally susceptible. This resistance was due (in most cases) to a single gene difference between the known strain and the new isogenic-resistant strain. Using these invaluable stocks, Snell (1958, 1959) has made considerable advances in knowledge of the genetics of transplantation antigens in mice, and has identified various different allelic genes at each of three loci concerned in histocompatibility, *H*-1, *H*-2 and *H*-3. Other workers in this field have also found Snell's lines extremely valuable, e.g., Hoecker *et al.* (1954) working on red-cell agglutination, and Bayreuther and Klein (1958) studying genetic variation in transplanted tumours.

III. Sources of Stocks

Suitable genetic stocks of mice may either be prepared by the interested worker himself or obtained from another laboratory. Preparation of a stock in some cases requires several generations of breeding and, since each generation takes about three months, it is usually better to make use of stocks existing already. To do this it is necessary to know where they may be obtained, and this again requires knowledge of the literature on this point.

The main sources of information are: *The Genetics of the Mouse* by H. Grüneberg (The Hague: Martinus Nijhoff. 1952); *An Annotated Catalogue of the Mutant Genes of the House Mouse* by H. Grüneberg (Medical Research Council Memorandum No. 33. London: H.M.S.O. 1956); *Standardized Nomenclature for Inbred Strains of Mice: Second Listing by The Committee on Standardized Genetic Nomenclature for Mice* (Cancer Research **20**: 145-169. 1960); *Mouse News Letter* (issued twice annually by the Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey, England); *Inbred Strains of Mice* (issued biennially by the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.).

Professor Grüneberg's *Genetics of the Mouse* gives detailed descriptions of the genetics and pathology of all mouse mutants known up to the time of its publication. His catalogue gives shorter descriptions of the pathology of mutants known up to a rather later date. These two publications are invaluable as a source of information on the types of mutant mice available.

The Mouse News Letter provides information on the location of mutants as well as on their existence. It is not a publication but an informal document circulated privately. Its purpose is to provide an exchange of information among those working with mutant stocks and inbred strains of mice, on the stocks that they carry and their research news. Contributing laboratories are asked to send full lists of the stocks that they carry every two years, with amendments only in the intervening issues. It lists the names and symbols of all mouse mutant genes currently known together with, in some issues, references to the original description of the discovery of the mutant and, in other issues, lists of the laboratories which have reported holding stocks. The research news includes brief reports of the discovery of mutants, investigations of their pathology and of their linkage relations. Those wishing to obtain or dispose of rare stocks may advertise the fact in the "Stock Exchange" section.

Inbred Strains of Mice is a companion document to the *Mouse News Letter* and, like it, is circulated privately. Contributing laboratories send in lists of their inbred strains only. The first issue appeared in 1959 and the information sent in by contributors to this issue was used to prepare an index list of inbred strains, their particular properties, and the laboratories holding them. This list is included in *Standardized Nomenclature for Inbred Strains of Mice.*

IV. Maintenance of Special Genetic Stocks

The previous paragraph describes how to locate a laboratory holding a suitable genetic stock. A very few of these laboratories will be prepared to sell mutant mice in sufficiently large numbers for experimental use. Most other laboratories will not be able to supply animals in such quantity, but will be willing to provide a small number of breeding pairs, so that the experimenter may raise mice for his own use. It will thus be essential to understand the special genetic techniques used in maintaining stocks of mutant mice. The remainder of this section will attempt to deal with the difficulties and complexities that may be met, so it may be as well to point out here that many stocks of mutant genes are no more difficult to maintain than normal stocks. It is only those with special difficulties that require mention here.

A. GENERAL

1. Size of stock

One of the first questions to be considered in maintaining any stock of animals is the amount of cage-space that must be given over to the stock in order to obtain the numbers of young needed for the experiment. Mutant stocks may require a considerably larger outlay of cage-space for a given return than normal stocks, for two reasons. (*a*) The animals may breed less vigorously than normals. (*b*) In some instances only a proportion of the young produced (e.g., one-quarter or one-half) will be of the desired genetic type. In the sub-sections below an attempt will be made to indicate what this proportion is likely to be in the various instances.

2. Recording

Breeding records for special genetic stocks of mice are more complex than those for normal stocks since, in addition to all the usual details, they should include records of the genes carried by the parent mice and their various offspring. This involves mastering the use of simple genetical symbols. In this chapter (and generally in mouse genetics) recessive mutant genes are denoted by symbols beginning with a small letter, e.g., *dw*, *dy*, *hr*; those with a visible effect in heterozygotes are given a symbol beginning with a capital letter, e.g. *T*, *Tw*, *Sd*; and normal genes are denoted by the + sign. (In some publications the normal gene corresponding to a particular mutant is designated, not by a + sign but by the same symbol as the mutant, beginning with a letter of the opposite case, e.g., *Dy*, the normal allele of *dy*, and *w*, the normal allele of *W*.)

When all the parent animals of a stock are homozygous for the same genes, e.g., an albino hairless stock *cc hrhr*, all the young will be expected to be likewise, and the genes carried need not be noted for each individual. When only a proportion of the animals are mutant, however, it is essential to record

the genes carried for each individual. The parents' records should state whether they are homozygous, heterozygous, or only possibly heterozygous for the mutant, and all the young should be classified as mutant or normal, at the earliest convenient age. This will indicate the proportion of mutant young being obtained. One suitable system of recording is explained by Carter (1957).

3. Inbreeding versus random breeding

An important question in maintaining a special genetic stock is whether to inbreed or not to inbreed. The next chapter explains how inbreeding over successive generations leads gradually to homozygosis of all the genes an animal carries and hence to great uniformity and stabilization of the important characteristics of the stock, such as tumour incidence. It also explains how, when inbreeding is first begun in a stock which has previously been random bred, the general quality of the stock, including its fertility, litter size, rearing of young, growth rate, etc., are likely to decline seriously over the first few generations as a result of inbreeding depression. After these first few generations no further depression is likely to occur.

Thus, in making the decision whether to inbreed or not, one has to weigh two factors: the advantages of inbreeding in leading to uniformity and stabilization, and its disadvantages in leading to inbreeding depression. In particular situations inbreeding may be obligatory, strongly advisable, or not advisable: (*a*) Inbreeding is obligatory when the conditions of the experiment require uniform animals with no genetic differences other than those involving the mutant in question. (*b*) Inbreeding is strongly advised when the stock is already highly inbred when supplied. Continued inbreeding will cause no further depression and will maintain the valuable homozygosis. (*c*) Inbreeding is not advised when only a small colony is to be maintained of a stock not previously inbred. Inbreeding depression could cause the loss of the stock.

Dangers to be guarded against are inadvertent inbreeding of a small stock through mating related animals, and the unintentional formation of separate lines when deliberately inbreeding a large stock. This second point is dealt with more fully in the next chapter.

The next chapter also deals with methods of inbreeding, the most convenient for special genetic stocks being brother × sister mating or repeated crosses to a standard inbred strain. Random breeding in this context is not truly random, since the mating of close relatives (e.g., full sibs, half-sibs, parents and offspring) should be avoided.

B. PARTICULAR TYPES OF MUTANTS

1. Dominant mutants with viable, fertile heterozygotes or homozygotes

Mutants referred to as dominant in this chapter are those which produce some easily visible effect when heterozygous. They may produce some much

more marked effect when homozygous, and hence are not true dominants, but for practical purposes it is convenient to distinguish genes of this type from true recessives. Very few truly dominant mutant genes are known in the mouse.

Since the effects of dominant genes can be seen in heterozygotes they are very simple to maintain.

TABLE III

Terms used in this chapter for various types of cross

Term	Recessive gene (*m*)	Dominant gene (*M*)
Outcross or cross	$++ \times mm$	$M+$ or $MM \times ++$
Intercross	$+m \times +m$	$M+ \times M+$
Backcross	$+m \times mm$	$M+ \times ++$ or MM
Homozygous mating	$mm \times mm$	$MM \times MM$

The usage may differ from that in other publications.

a. Animals carrying the dominant can be crossed (Table III) to any normal stock. Half the offspring will then be heterozygotes and the rest normal (Fig. 1A). The heterozygotes will make parents for further crosses of the same kind. This type of mating can be used to give litter-pairs of mutant and normal animals, if the experiment should require these, and also to make an inbred stock of the mutant coisogenic with an inbred strain, e.g., *Standardized Nomenclature* lists strains C57BL/10-*lu* and C57BL/10-*lx*, made by crossing the genes *lu* and *lx* (used as dominants) repeatedly on to strain C57BL/10.

b. If mutant homozygotes are wanted, heterozygotes should be mated together, e.g., Twirler, $Tw+ \times Tw+$ (Fig. 1B). One quarter of the offspring will then be homozygotes for use ($TwTw$), half will be heterozygotes ($Tw+$) which can be used for further matings of the same type, and the remainder will be normals ($++$). The three types will be distinguishable by the gene effect.

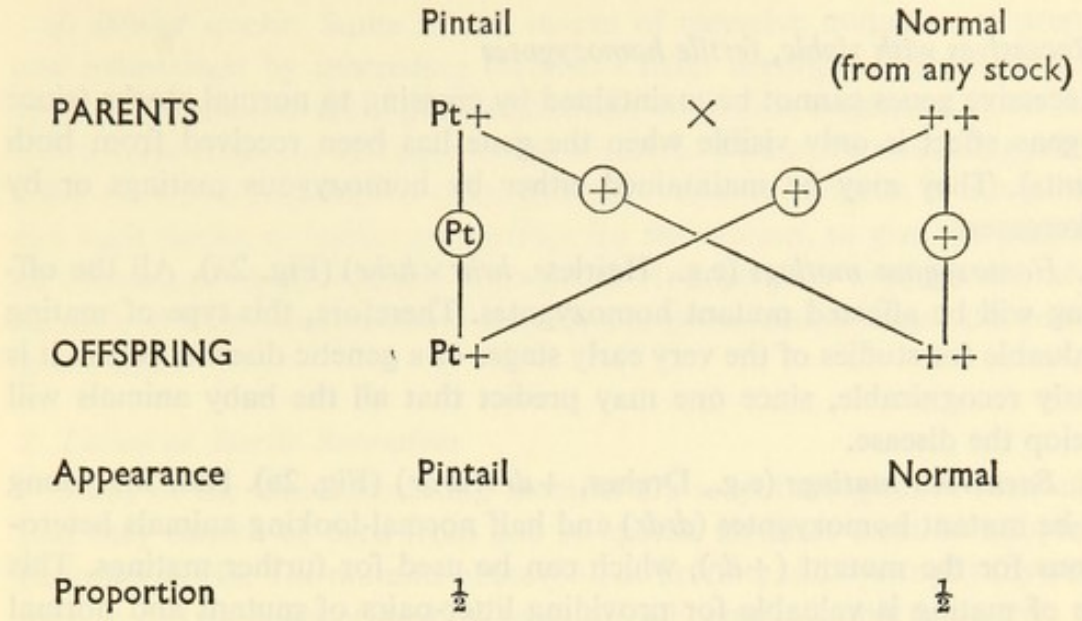
c. If the homozygotes are viable and fertile they can be mated together to give a stock in which all the young will be mutant homozygotes. Very few dominant mouse mutants come into this category; examples are rex, *Re*, caracul, *Ca*, and sombre, *So* (Bateman, 1961).

d. Several dominant genes may be maintained in the same stock if desired. Providing the genes are not linked, the young will carry them in all possible combinations. The stock can be maintained by mating animals of complementary types, e.g., in *Mouse News Letter* 25, Harwell lists a stock LI carrying the genes *lx*, *Ra*, *Re*, *Sd*, W^v. This can be maintained by matings:

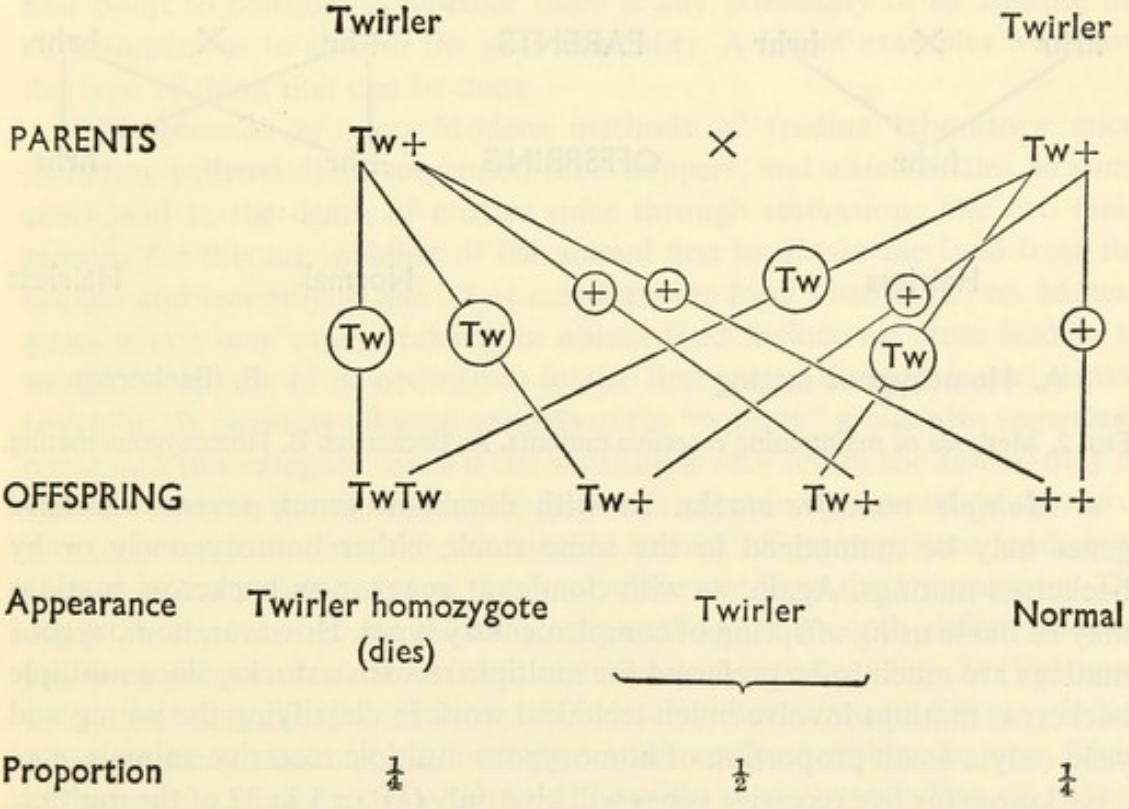
$+lx\ Ra+\ Re+\ Sd+\ W^v+ \times ++\ ++\ ++\ ++\ ++$
$+lx\ Ra+\ Re+\ Sd+\ ++ \times ++\ ++\ ++\ ++\ W^v+$
$+lx\ Ra+\ ++\ ++\ W^v+ \times ++\ ++\ Re+\ Sd+\ ++$

etc.

(The genes *lx* and W^v and *Ra* and *Sd* are linked, but this does not alter the possible types of mating.)



A. Cross to a normal stock



B. Intercross of mutant heterozygotes

FIG. 1. Methods of maintaining dominant mutants.

2. Recessives with viable, fertile homozygotes

Recessive genes cannot be maintained by crossing to normal stocks (since the gene effect is only visible when the gene has been received from both parents). They may be maintained either by homozygous matings or by backcrosses.

a. Homozygous matings (e.g., Hairless, *hrhr* × *hrhr*) (Fig. 2A). All the offspring will be affected mutant homozygotes. Therefore, this type of mating is valuable for studies of the very early stages of a genetic disease, before it is grossly recognizable, since one may predict that all the baby animals will develop the disease.

b. Backcross matings (e.g., Dreher, $+dr \times drdr$) (Fig. 2B). Half the young will be mutant homozygotes (*drdr*) and half normal-looking animals heterozygous for the mutant ($+dr$), which can be used for further matings. This type of mating is valuable for providing litter-pairs of mutant and normal animals.

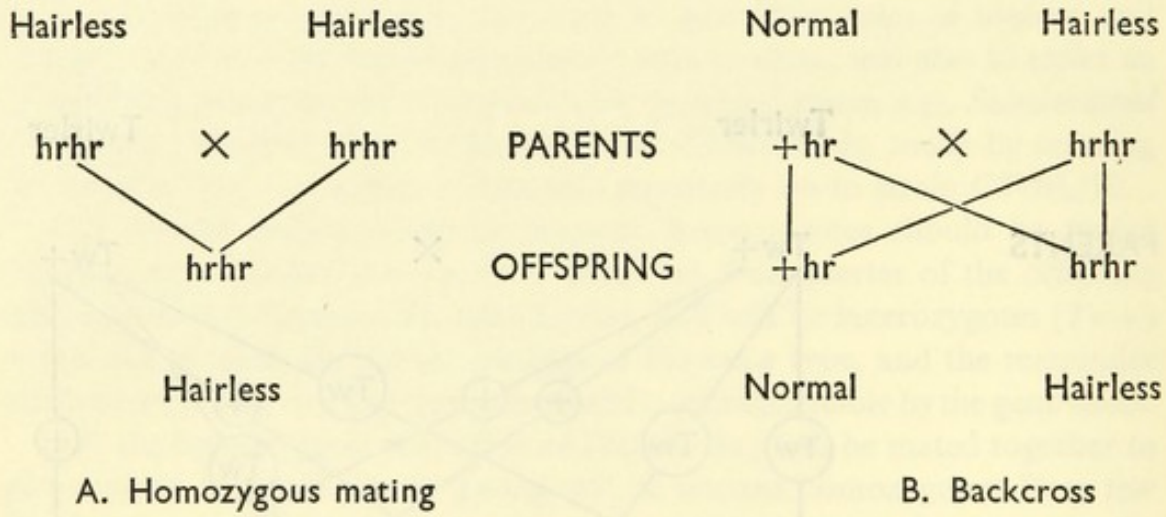


FIG. 2. Methods of maintaining recessive mutants. A. Backcross. B. Homozygous mating.

c. Multiple recessive stocks. As with dominant genes, several recessive genes may be maintained in the same stock, either homozygously or by backcross matings. Again, as with dominant genes, new backcross matings may be made using offspring of complementary types. However, homozygous matings are much to be preferred for multiple recessive stocks, since multiple backcross matings involve much technical work in classifying the young and yield only a small proportion of homozygous multiple recessive animals, e.g., a backcross for five recessive genes will give only $(\frac{1}{2})^5$ or 1 in 32 of the multiple recessive type. The preparation of homozygous multiple recessive stocks takes many generations of breeding and hence they are greatly cherished once prepared. They are much used in experiments on mutation, e.g., the T stock (Oak Ridge) *aa bb* $c^{ch}c^{ch}$ *dd pp ss sese.*

d. Inbred stocks. Some inbred stocks of recessive mutants are prepared and maintained by inbreeding (brother × sister mating) offspring of homozygous or backcross matings described above. Others, isogenic with an inbred strain, have arisen by mutation of the recessive within the strain, e.g., CBA-*p* CBA-*se*, C57BL-*b*, RIII-*ro* (*Standardized Nomenclature*). It is advisable to run such stocks as backcross matings for the mutant, to give the possibility of obtaining normal/mutant litter-pairs. Homozygous matings can always be derived from backcrosses if they should be needed, whereas the converse is not true.

3. Lethal or Sterile Recessives

Many of the most interesting mutants are so pathological in their effects that they cannot be bred from and so special methods must be adopted to maintain stocks. The mutants obese (*ob*), dwarf (*dw*) and dystrophy (*dy*), much used in certain fields of medical research, all come into this category.

The special methods for maintenance may involve changes either in the environment of the animals or in the breeding procedure.

a. Change of environment. When faced with the problem of maintaining a recessive mutant which is either lethal or sterile when homozygous the first point to consider is whether there is any possibility of so altering the environment as to abolish the gene's lethality. A list of examples will show the type of thing that can be done.

i. Modification of diet. Modern methods of feeding laboratory mice, including pelleted diets, suspended food-hoppers, and water bottles, in some cases lead to the death of mutant mice through starvation. The two main reasons for this are inability of the animal first to obtain the food from the hopper and secondly to bite off or masticate the food when obtained. Mutant genes which may cause inability to obtain food include all those leading to weakness or lack of co-ordination in the limbs, e.g., dystrophy (*dy*), ataxia (*ax*) (Fig. 3). Severely affected animals of the "waltzer" group also sometimes come into this category, since if the waltzing is very severe the animal may be unable to stay in the same place long enough to get food. Animals with neuromuscular weakness may also be unable to bite off or masticate food, even after they have obtained it, if the weakness extends to the jaw muscles. The other obvious reason for inability to masticate is a tooth defect. Mutant genes resulting in absence or defect of the incisors include tabby, *Ta* (Falconer, 1953), and downless, *dl* (Phillips, 1960b), while homozygotes for grey-lethal, *gl*, and microphthalmia, *mi*, have the molars retained within the jaws. These animals must be fed powdered food, possibly made by grinding up pellets, in a suitable container. Very weak animals may be unable to drink from the water bottle, even when this is provided with a specially long spout. These should be fed a wet mash. Of course, animals permanently as weak as this are most unlikely to breed and be of use in maintaining a stock. However, it is

quite common for mutant animals to need wet-mash feeding in the first 1-2 weeks after weaning, and to graduate to normal feeding later as their limbs and jaws strengthen through growth. Phillips describes how downless mice were kept alive and proved fertile by wet-mash feeding.

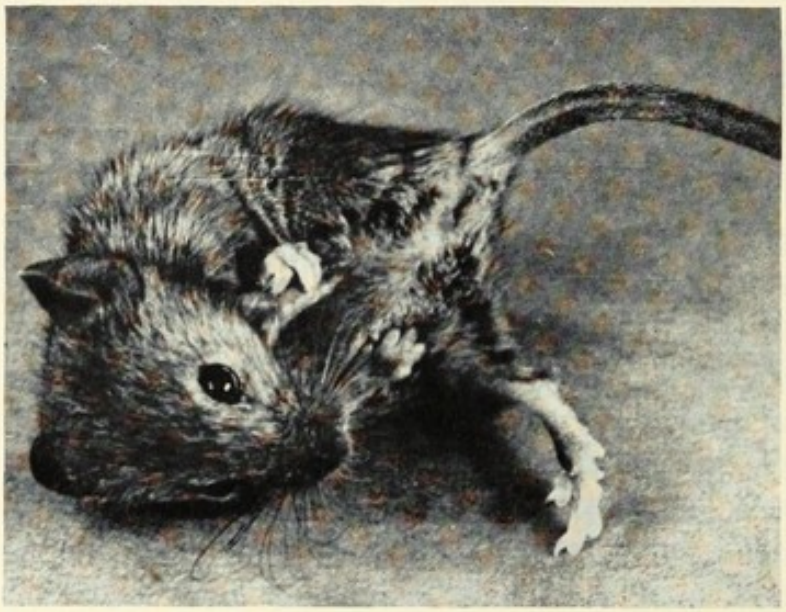


FIG. 3. Homozygous ataxic mouse (*axax*), showing the animal's weakness and consequent inability to obtain food. It cannot right itself. (Photograph originally published in *J. Hered.*)

A second type of modification of diet is restriction of intake. As described above, animals homozygous for the gene obese, *ob*, are very fat, and sterile. Lane and Dickie (1954) showed that restriction of the calorie intake would prevent the obesity and that males so treated were fertile, though still of course genetically obese. This method is now used at Bar Harbor to obtain fertile males for the routine maintenance of *ob* (Russell, 1960).

ii. Modification of nest or bedding. Animals lacking hair, e.g., hairless *hr*, (Fig. 4), and those with sparse hair, e.g., ragged, *Ra*, and fuzzy, *fz*, lose heat and moisture from the body more rapidly than normal animals, and may need to be supplied with extra bedding material or a nest-box. As with special feeding, mutants are much more likely to need this attention in the first one or two weeks after weaning, than when fully adult.

Animals afflicted with mutants of the "waltzing "type have great difficulty in maintaining a suitable nest, since they cannot help waltzing over it as soon as it is constructed. A nest-box or nest-guard is often completely successful against this problem (Fig. 5).

iii. Fostering young or removing sire. Females homozygous for certain mutants are fertile but do not rear young, even when provided with a nest-box. They may yet be used for breeding if the young are removed at birth and

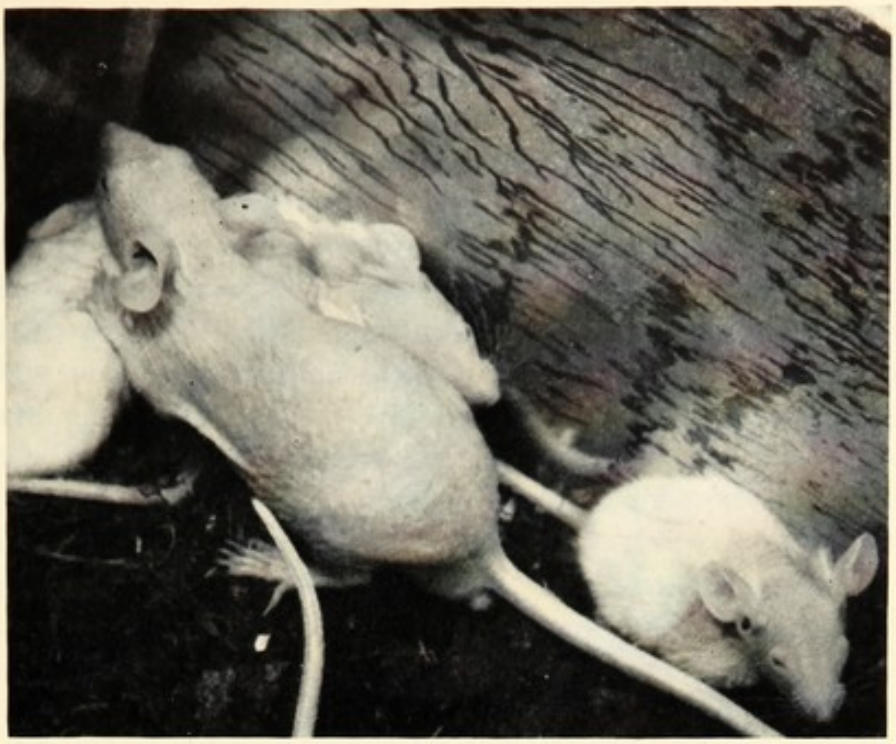


FIG. 4. Albino hairless mouse (*cc hrhr*) and three-week-old litter.

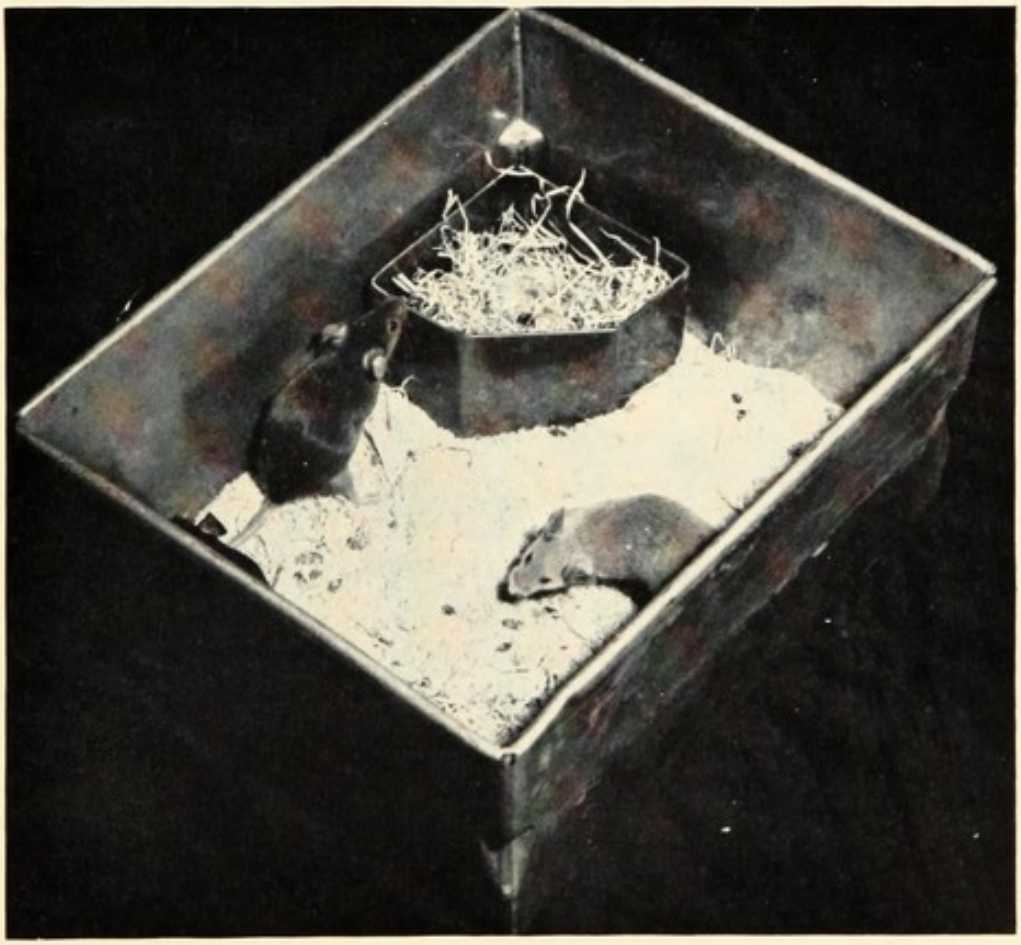


FIG. 5. Nest-guard in use to protect a litter from its dreher (*drdr*) sire, seen circling in the foreground.

placed with a foster mother. The foster mother will recognize the difference in scent of the fostered young and may at first reject them. To maximize the chances of success in fostering, the litters exchanged should be as nearly as possible the same age, and the number of young that the foster mother is expected to rear should be near to that in her natural litter, some or all of her own litter being removed. Providing a clean cage or fresh nesting material at the time of fostering may also help, by distracting the female's attention from the young until she has become accustomed to the strange scent.

In other cases failure to rear young may be due to their being attacked by the mutant sire. "Waltzing" mutants are prone to this difficulty, which can be dealt with by removing the male before a litter is due.

iv. Hormone treatment. Genetically sterile animals can sometimes be induced to become pregnant by injection of appropriate hormones. Smithberg and Runner (1957) induced mating in females homozygous for obese (*obob*) by two intraperitoneal injections of 3 i.u. pregnant mare's serum on consecutive days, followed by 2 i.u. human chorionic gonadotrophin on the afternoon of the 3rd day. The females were then placed with males, and in those which mated pregnancy was maintained by daily injections of 2·5 mg progesterone in 0·1 ml oil, up to day 18 of pregnancy. Fowler and Edwards (1961) used a similar technique with females homozygous for a gene *midget*. However, Smithberg and Runner considered this treatment unsatisfactory for routine maintenance of stocks of obese. With a new type of genetic dwarf mouse Schaible and Gowen (1961) were able to induce fertility in males by injections of growth hormone, but this work is still in an early stage.

b. Genetic testing for heterozygotes. When it is impossible to obtain fertile mutant homozygotes, the stock must be maintained by mating together heterozygous animals, with the result that on the average one-quarter of the offspring will be the desired recessive homozygotes (Fig. 6). Suppose that a

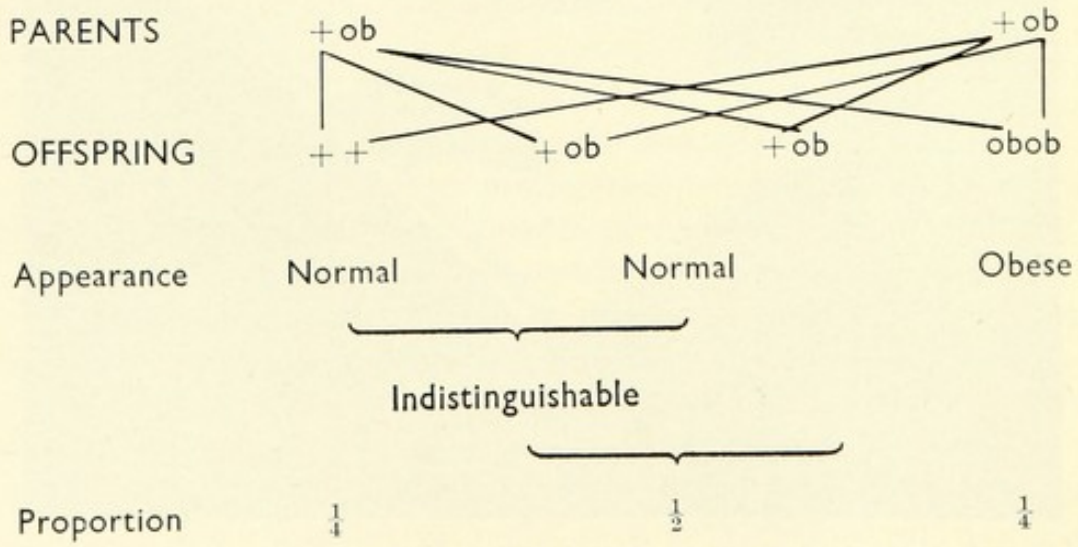


FIG. 6. Progeny resulting from an intercross mating for a recessive gene.

stock of this kind is started by obtaining heterozygotes from another laboratory. Difficulty first arises when it is necessary to find new heterozygous parents to replace old ones. One-third of the normal offspring of the original pairs are not heterozygotes, but homozygous for the normal gene. The two types can only be distinguished by genetic test. The procedure is therefore to put up several pairs of normal-looking offspring from heterozygous matings and observe which pairs produce homozygous mutant young. These pairs are then known to be heterozygous and kept, and all other pairs are discarded. Fig. 7 shows that on the average four-ninths of pairs tested are expected to

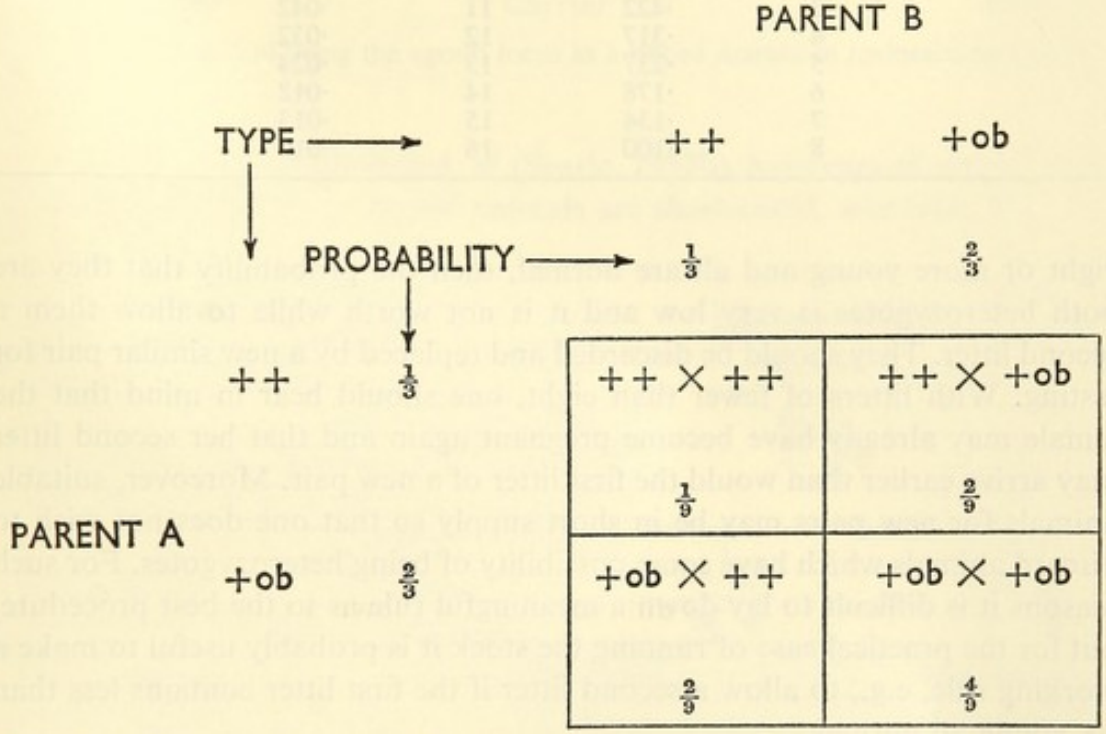


FIG. 7. Possible types of cross resulting from matings between the normal-looking offspring of Figure 6. Only the type $+ob \times +ob$ will produce mutant young.

be of the desired type $+m \times +m$, the discarded ones being four-ninths of the type $++ \times +m$, and one-ninth $++ \times ++$. Thus, one must put up nine-fourths of the number of new pairs actually wanted and must allow for this in deciding the number of cages required to maintain the stock. There is also the problem of how many litters with no mutants a pair should be allowed to have before it is discarded. Since only one-fourth of the young are of the recessive type, some genuinely heterozygous parents will have no mutants in their first litter. If there are n young the probability that all will be of the mutant type is $(\frac{1}{4})^n$, and that all will be of the non-mutant type is $(\frac{3}{4})^n$. The values of $(\frac{3}{4})^n$ for varying n are given in Table IV. From this it can be seen that the probability that a pair of heterozygotes should produce a litter of ten

young all non-mutant is as low as ·056, and even a litter of eight normals has a probability of only 0·10. Clearly, if the first litter of a pair under test contains

TABLE IV

The probability, for values of n, *of a pair of heterozygous animals producing* n *young, all normal*

n	Probability $=(\frac{3}{4})^n$	n	Probability $=(\frac{3}{4})^n$
1	·750	9	·075
2	·563	10	·056
3	·422	11	·042
4	·317	12	·032
5	·237	13	·024
6	·178	14	·018
7	·134	15	·013
8	·100	16	·010

eight or more young and all are normal, then the probability that they are both heterozygotes is very low and it is not worth while to allow them a second litter. They should be discarded and replaced by a new similar pair for testing. With litters of fewer than eight, one should bear in mind that the female may already have become pregnant again and that her second litter may arrive earlier than would the first litter of a new pair. Moreover, suitable animals for new pairs may be in short supply so that one does not wish to discard animals which have some possibility of being heterozygotes. For such reasons it is difficult to lay down a meaningful rule as to the best procedure, but for the practical ease of running the stock it is probably useful to make a working rule, e.g., to allow a second litter if the first litter contains less than six young all normal.

Mutant genes that are normally maintained by this method include dwarf (*dw*), ichthyosis (*ic*) and grey-lethal (*gl*). It is obvious that the proportion of mutant young obtained from a stock maintained in this way is very small; four-ninths of the pairs produce one-fourth of mutant young, and the rest none. Therefore this method should be avoided wherever possible.

c. Use of linked marker genes. The procedure described in the last paragraph, though unavoidable for many mutants, is extravagant both in cage-space and in requirements for technical care. Therefore, any means by which heterozygous animals can be distinguished from normals without genetic testing is very valuable. One possibility is to make use of marker genes closely linked to the mutant. An example should make this clear. The mutant kreisler, *kr*, is closely linked to the agouti locus. Fig. 8 shows a mating scheme by which offspring carrying *kr* can be distinguished from homozygous normals by their agouti phenotype. Similarly, in the second example, offspring

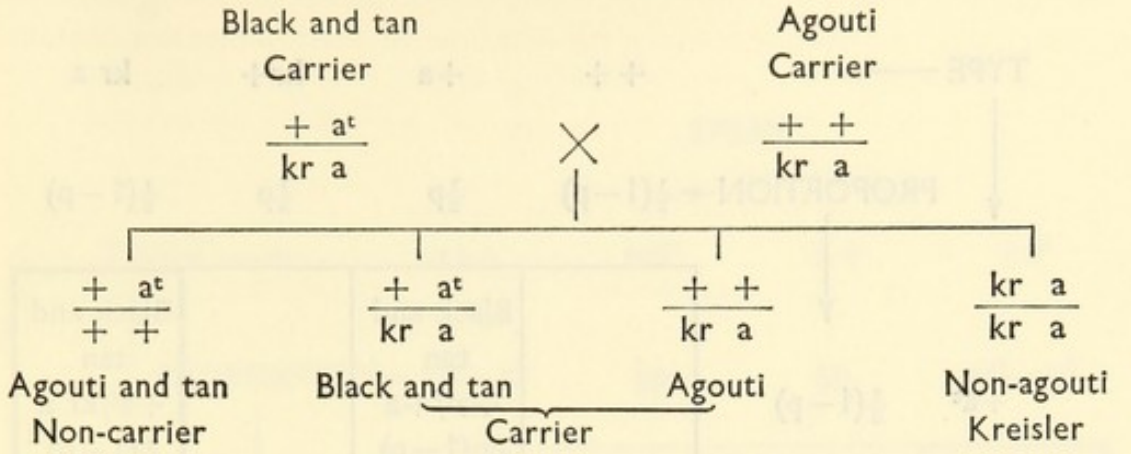


FIG. 8. Method of using the agouti locus as a linked marker in maintaining kreisler.

heterozygous for dilute-lethal, d^l (Searle, 1952b), have ears of normal length while the homozygous normal animals are short-eared, *sese* (Fig. 9).

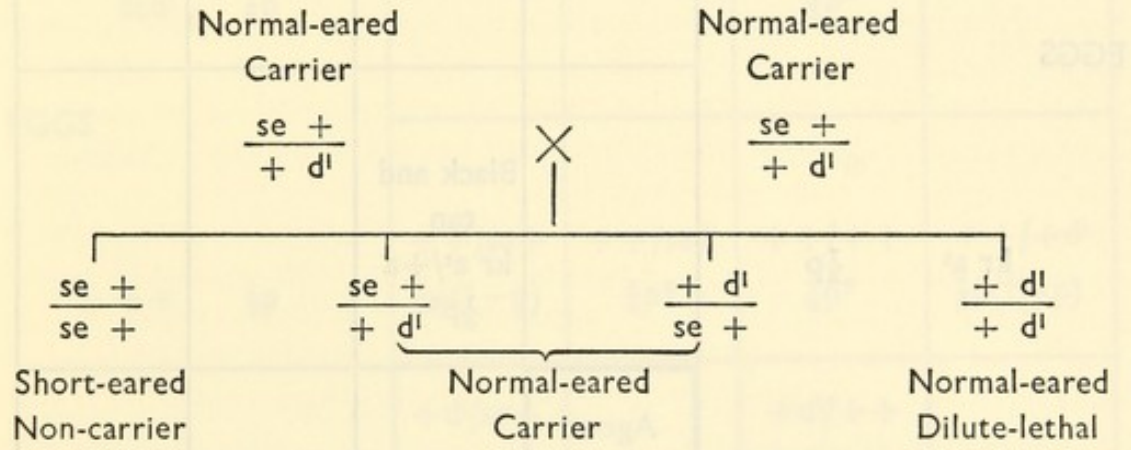
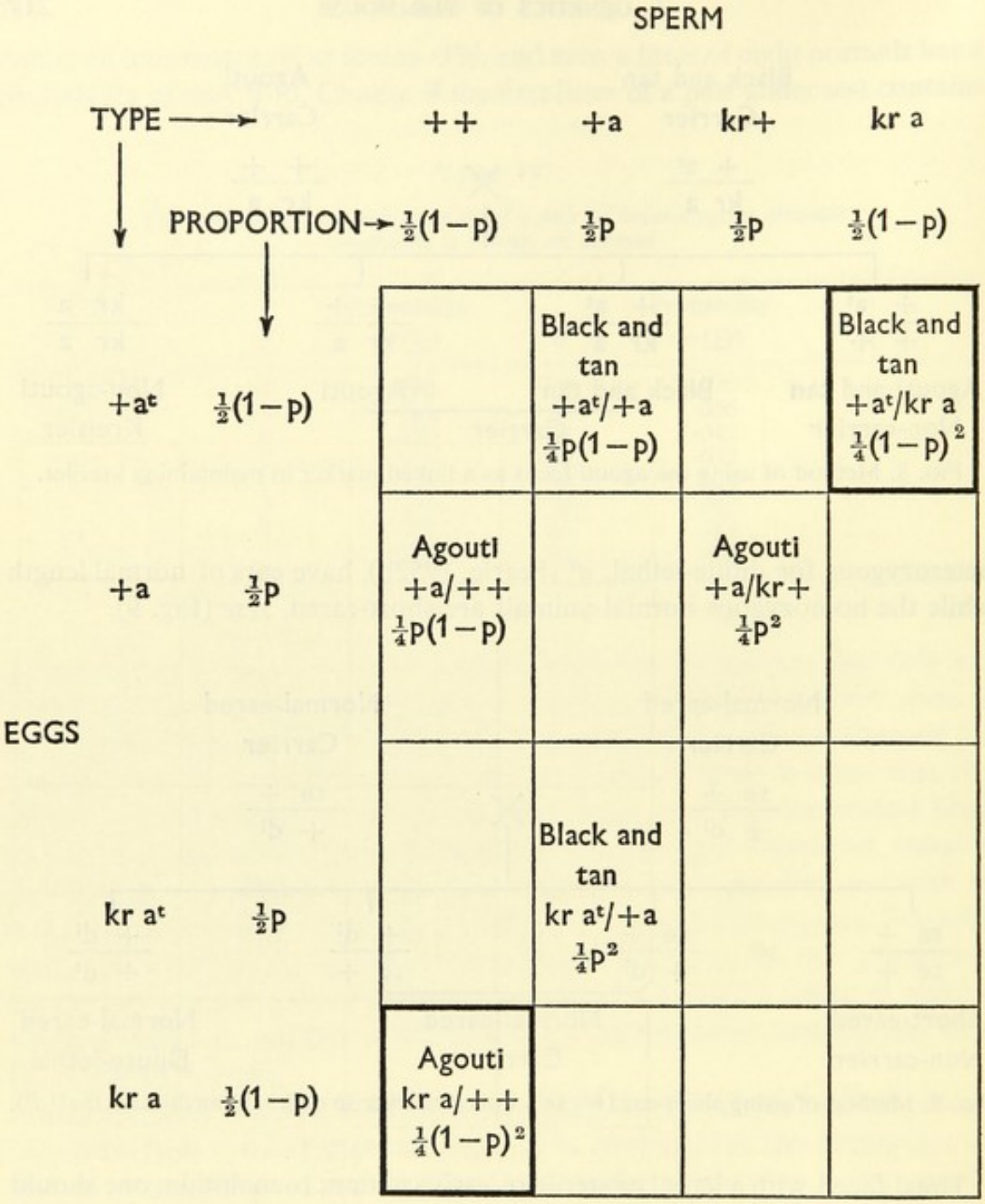


FIG. 9. Method of using short-ear (*se*) as a linked marker in maintaining dilute-lethal (d^l).

Thus, faced with a lethal or sterile recessive mutant to maintain, one should consult recent genetic literature (e.g., *Mouse News Letter*) to find whether it is known to be linked to a good marker gene. The marker must be a gene with easily recognizable effects, but not itself a problem to maintain. It is also important that its linkage with the mutant should be a close one, since otherwise the mutant can be lost as a result of genetic crossing-over between it and its marker. The calculations in Fig. 10 show that in the case of kreisler and its agouti-locus marker the proportion of marked animals that are of the desired genetic type is approximately $(1\text{-}p)$, where p is the recombination fraction. In this instance p is 3% and hence 97% of these animals will be of the correct type. In the case of dilute-lethal and short-ear the proportion of non-short-ear animals of the correct type is approximately $(1-p)^2$ (Fig. 11). For these two



Total animals of correct agouti colour $= \frac{1}{2}(1-p+p^2)$

Total animals of correct genetic type $= \frac{1}{2}(1-p)^2$

If p is very small, p^2 can be neglected

Hence, proportion of marked animals

that are of correct genetic type $\simeq \frac{(1-p)^2}{1-p}$

$= 1-p$

FIG. 10. In a cross of the type shown in Figure 8, the proportion of marked animals that will be of the correct genetic type if a fraction p of the gametes are crossovers and $(1-p)$ are non-crossovers.

SPERM

TYPE →	se+	sed^l	++	+d^l
PROPORTION →	$\frac{1}{2}(1-p)$	$\frac{1}{2}p$	$\frac{1}{2}p$	$\frac{1}{2}(1-p)$

EGGS					
se+	$\frac{1}{2}(1-p)$			se+/++ $\frac{1}{4}p(1-p)$	se+/+d^l $\frac{1}{4}(1-p)^2$
sed^l	$\frac{1}{2}p$			sed^l/++ $\frac{1}{4}p^2$	
++	$\frac{1}{2}p$	++/se+ $\frac{1}{4}p(1-p)$	++/sed^l $\frac{1}{4}p^2$	++/++ $\frac{1}{4}p^2$	++/+d^l $\frac{1}{4}p(1-p)$
+d^l	$\frac{1}{2}(1-p)$	+d^l/se+ $\frac{1}{4}(1-p)^2$		+d^l/++ $\frac{1}{4}p(1-p)$	

Total normal-eared non-dilute animals $= \frac{1}{4}[2(1-p)^2+4p(1-p)+3p^2]$
$= \frac{1}{4}(2+p^2)$

Total such animals of correct genetic type $= \frac{1}{2}(1-p)^2$

If p is very small, p^2 can be neglected

Hence, proportion of marked animals that are of correct genetic type $\simeq \frac{2(1-p)^2}{2}$
$= (1-p)^2$

FIG. 11. Calculations similar to those of Figure 10 for the cross shown in Figure 9.

genes p is 0·16%, but suppose that in some other similar case p were as large as 10%. Then the proportion $(1-p)^2$ would be 0·81, and therefore the chance of any pair of chosen animals both being of the right type would be only $0{\cdot}81^2$ or 0·66. Thus, about one pair in three chosen in this way would not be of the correct type, and it would be necessary to watch for these pairs and discard them, as in the previous method. This does not mean that the use of a linked-marker is valueless in such a case. Without the marker only four-ninths of all pairs tested would prove themselves to be heterozygous, whereas with a marker showing 10% crossing-over nearly two-thirds will do so, and there will thus be less wastage of cage-space. It is now clear that linkages allowing appreciably more than 10% crossing-over will confer relatively little assistance, and that below this figure the closer the linkage the better.

A particular class of mutants for which the linked-marker method is valuable are the sex-linked mutants. Many sex-linked mutants of the mouse are either lethal or sterile in the male and hence must be maintained by matings of heterozygous females. Half the male offspring of these heterozygotes will show the mutant and half the female offspring will be carriers suitable for maintaining the stock. Some sex-linked genes exert a visible effect in the heterozygotes, which can easily be picked out (e.g., mottled, *Mo*; dappled, Mo^{dp}; and tabby, *Ta*), but others do not, e.g., jimpy, *jp*; scurfy, *sf*. In these latter cases one of the viable, fertile sex-linked genes can be used as a linked marker to enable the heterozygotes to be identified. Fig. 12 shows a

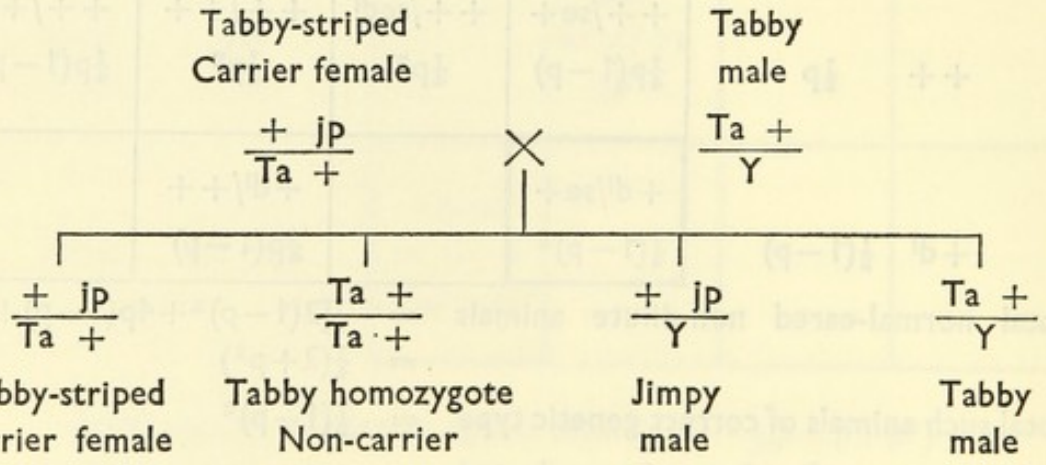


FIG. 12. Method of using the linked marker tabby (*Ta*) in maintaining the sex-linked recessive jimpy (*jp*).

suitable mating scheme using tabby as a linked marker for jimpy. Blotchy, *Blo*, (L. B. Russell and Saylors, 1962) is another suitable marker for sex-linked genes.

d. Balanced-lethal method. This is a special case of the linked marker method. In the previous example using short-ear and dilute-lethal, suppose that in place of short-ear we had a gene which when homozygous was lethal

before birth. Then only two types of young would be born: the desired mutant homozygotes, and double heterozygotes suitable for continuing the stock. The principle is that if there are two recessive lethal genes closely linked to each other, heterozygotes having these two genes on opposite chromosomes will breed true, i.e., their only surviving offspring will be genetically like themselves. The lethals are said to be balanced against each other.

This method was used by L. B. Russell and W. L. Russell (1960) for the maintenance of a lethal agouti allele, a^x. The gene A^y at the same locus results in a yellow coat when heterozygous and acts as an early embryonic lethal when homozygous. If heterozygotes of the type A^y/a^x are mated together, both the A^yA^y and the a^xa^x homozygotes will die, and all the surviving offspring will be A^y/a^x and thus suitable for maintaining the stock.

The example of balanced lethals best known to mouse geneticists is that of the *t*-alleles. The mutant gene *T* results in a shortened tail when heterozygous (*T*+) and is lethal before birth when homozygous. A series of recessive mutants, which show no recombination with *T*, and are called *t*-alleles, are again lethal before birth when homozygous, and when in compound with *T* (*Tt*) result in taillessness (as opposed to the merely shortened tail of *T*+). Thus *Tt* animals when mated together produce three types of embryos, *TT*, *tt* and *Tt*, of which only the *Tt* survive. Such tailless stocks therefore breed true, and the *t*-alleles can be maintained in them with the minimum of labour in classifying young and selecting suitable animals for breeding (Sinnott *et al.*, 1958).

The genes for ataxia, *ax*, and twirler, *Tw*, provide a further example of a balanced-lethal system. Animals homozygous for ataxia develop severe tremor and paralysis and are unable to breed. The gene is closely linked to twirler, *Tw*, which when heterozygous results in waltzing behaviour due to an inner ear defect, and when homozygous causes death through harelip and cleft palate (Lyon, 1958). Animals of type *Tw*+/+*ax* mated together will produce two types of surviving young: *axax* homozygotes for experimental use, and further *Tw*+/+*ax* young with which the stock can be maintained.

It is important to note that, although all the young obtained from a balanced lethal stock are suitable for breeding or use, this does not mean that the absolute number of suitable young is higher. The number of embryos formed at fertilization will be approximately the same as in normal mice but half (in the *t*-allele example) or a quarter (as in the *Tw-ax* example) will die, so that at birth the litter will contain a correspondingly reduced number of young, i.e., the absolute number of suitable animals is the same as in stocks using a viable linked marker. The advantage of the balanced lethal method lies in reducing the labour of classifying and selecting the correct types of young. As with the linked marker method, crossing-over between the two balanced lethals reduces the efficiency of the method. In the examples quoted crossing-over occurs only rarely (1% or less).

e. Ovarian transplantation. By this technique ovaries from females which cannot breed because they are homozygous for a recessive mutant gene are transplanted into a normal female. This animal is then mated with a male heterozygous for the mutant. She will give birth to young derived from the *mutant* ovaries. Thus, on the average, half of them will be mutant homozygotes, and the remaining half known heterozygotes suitable for maintaining the stock.

This method was originally described by Robertson (1942), and the technique was further developed by W. L. Russell and Hurst (1945) using adult ovaries, and by W. L. Russell and Douglass (1945) using embryonic ovaries. This latter method, with embryonic ovaries, was then used by W. L. Russell and E. S. Russell (1947) and W. L. Russell and Gower (1950) to investigate genetic problems. (Russell and Russell implanted normal ovaries into genetically sterile females homozygous for the gene W^v, which then bore young. This was of course of no use for maintaining a stock of W^v since the implanted ovaries did not carry the gene.) The technique for adult ovaries was later again modified by Stevens (1957) and has since been extensively used by Stevens *et al.* (1957) for routine maintenance of the dystrophy gene *dy*. Parrott and Parkes (1956) used X-irradiation to sterilize the host's own ovaries, rather than surgical removal, and further technical improvements have been suggested by Mussett and Parrott (1961) and by Jones and Krohn (1960).

For successful ovarian transplantation the donor and host females must be antigenically compatible and, in practice, this means that they must belong to the same inbred strain, or the host may be a hybrid between two inbred strains, one of which is that of the donor. Moreover, Russell and Hurst showed that after surgical removal, regeneration of host ovaries occurred quite frequently, and hence it is important that the young from donor and host ovaries should be distinguishable, e.g., by colour. Russell and Hurst, and Stevens used the inbred strain 129, which has been maintained heterozygous for two mutant alleles at the *c*-locus, c^{ch} and *c*. Fig. 13 (adapted from Russell and Hurst) shows how these alleles can be used for distinguishing young from donor and host ovaries. Mussett and Parrott (1961), on the other hand, used a CBA strain, CBA/Fa-a^t (known by them as G) which is heterozygous for the mutant a^t.

The technique of their operation is described by Jones and Krohn in detail. In brief, the ovaries of the host are pulled out through a dorsal incision, and removed *via* a slit in the ovarian capsule. A half or whole donor ovary is then placed inside the host ovarian capsule and the exteriorized structures are put back into the body cavity. Stevens found that a half-ovary gave better results than a whole or a quarter ovary. Mussett and Parrott agreed, finding that if the volume of grafted ovary was too large the ovarian capsule did not close and shed ova did not reach the Fallopian tube. They also found that the fertility of the graft was inversely related to the age of the donor. The radiation

dose which they gave to sterilize the host was at first 900r but in later experiments doses as low as 200r proved successful. Animals grafted immediately after irradiation were more fertile than those grafted after a delay of four weeks, and no young of host origin were born.

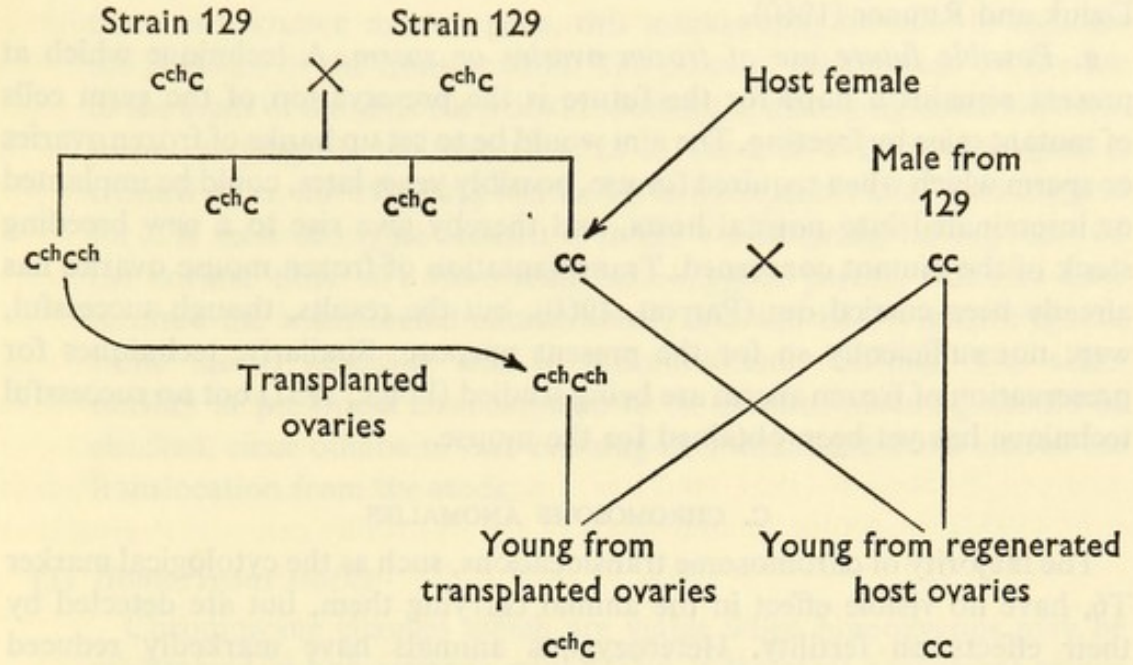


FIG. 13. The use of *c*-alleles in strain 129 for colour-marking young derived from donor and host ovaries after ovarian grafting.

Thus, in maintaining a mouse mutant by ovarian transplantation, the main limitation is that the method requires an inbred genetic background. With great good fortune the original mutation of the gene *dy* took place in strain 129, and hence the stock was suitable for ovarian transplantation with no further breeding. Any other mutants arising in this strain or in strain CBA will also obviously be suitable. Similarly, mutants arising in any other inbred strain may be maintained by transplanting mutant ovaries into normal females of the same strain, but it may prove impossible to arrange that the donor's young shall be colour marked. Then Mussett and Parrott's technique should be used, since they claimed that no young of host ovaries were born after X-ray sterilization. Jones and Krohn also found no young from host ovaries, but their data were less extensive. Mussett and Parrott's technique may again be tried when the mutant cannot be obtained on an inbred background, since they have obtained success using outbred and hybrid mice, although the results were very variable.

f. Artificial insemination. When mutant males are sterile through inability to mate, their sperm may be used for artificial insemination of heterozygous females. The technique is at present not economic for routine maintenance of a mutant, since the male must be killed to obtain sperm, which is only

sufficient for two or three inseminations. However, it has been used with the mutant *dy* for obtaining litters of mice all known to be homozygous *dydy*. Females bearing *dydy* ovarian grafts were artificially inseminated with sperm from *dydy* males (Wolfe and Southard, 1961). Techniques for artificial insemination of mice are described by Snell *et al.* (1944), Edwards (1957) and Dziuk and Runner (1960).

g. Possible future use of frozen ovaries or sperm. A technique which at present remains a hope for the future is the preservation of the germ cells of mutant mice by freezing. The aim would be to set up banks of frozen ovaries or sperm which when required for use, possibly years later, could be implanted or inseminated into normal hosts, and thereby give rise to a new breeding stock of the mutant concerned. Transplantation of frozen mouse ovaries has already been carried out (Parrott, 1960), but the results, though successful, were not sufficiently so for the present purpose. Similarly, techniques for preservation of frozen sperm are being studied (Polge, 1957) but no successful technique has yet been obtained for the mouse.

C. CHROMOSOME ANOMALIES

The majority of chromosome translocations, such as the cytological marker T6, have no visible effect in the animal carrying them, but are detected by their effects on fertility. Heterozygous animals have markedly reduced fertility because they pass on unbalanced chromosome sets to their offspring which then die as embryos. Such heterozygotes are said to be semi-sterile. At the present time, although many different mouse chromosome translocations are maintained by professional geneticists, the only one in use outside genetical laboratories is the marker T6. Therefore, T6 will be used here as an example in describing the various methods of maintaining such stocks.

The three main methods are:

(*1*) *Outcrosses of heterozygotes*

Animals known to be heterozygous for the translocation may be crossed to any normal stock. Half the offspring will again be heterozygous and the remainder normal. The two types will be indistinguishable visually, and in order to detect the heterozygotes the fertility of the animals must be tested. Methods of making fertility tests and criteria for semisterility are given by Carter *et al.* (1955). It must be emphasized, however, that these criteria apply only to the group of translocations actually studied by them. Translocations vary in their fertility and hence any new one might fall outside the range of fertilities of this group. T6 is of course one of the group, with a fertility in heterozygotes about 37% of normal, and hence the criteria are suitable for it.

It is obvious that this method is tiresome, since the heterozygotes, whether for breeding or for use, cannot be recognized without a

breeding test. Therefore one of the two following methods should be used whenever possible.

(2) *Use of linked markers*

When the point at which the chromosome change has occurred is close to a known marker gene, this marker may be used to indicate the presence of the translocation. The point of exchange in T6 is close to the locus of the gene for piebald spotting, *s*, the translocated chromosome carrying the normal allele of *s*. Thus, if a T6 homozygote is crossed to *ss*, the offspring will be T6 heterozygotes also heterozygous for *s*. If these are again crossed to *ss* the $+s$ offspring, having received the normal allele of *s* from their heterozygous parent, will also have received the translocated chromosome, and will be T6 heterozygotes, while the *ss* offspring will be chromosomally normal. The semi-sterility of presumed heterozygotes to be used for breeding should be checked, since otherwise rare crossing-over could cause the loss of the translocation from the stock.

(3) *Homozygous stocks*

Chromosome changes which are viable when homozygous may be maintained in homozygous stocks. This is the most convenient means of maintaining T6. Two different homozygous stocks of T6 have originated from Harwell, one non-inbred and the other coisogenic with strain CBA. These stocks may be maintained, as with homozygous stocks of recessive genes, by matings within them, all the offspring being homozygous for the translocation. If heterozygotes are needed for experimental use they may be obtained by crossing the homozygotes to any normal stock. These heterozygotes may be used for breeding as known heterozygotes if so desired, but their offspring should on no account be bred from, as they will not be known heterozygotes.

(4) *Maintaining stocks of XO mice*

Female mice lacking one X-chromosome, a type of abnormality discovered in mice by Welshons and Russell (1959), are sometimes used in non-genetic laboratories. Mice of the type XO are normal fertile females indistinguishable in appearance from normal XX animals. If the single X-chromosome carries a sex-linked mutant gene, however, the gene will express itself fully as in a male, so that XO females carrying a sex-linked mutant are distinguishable from XX females heterozygous for the mutant. The technique of maintaining stocks of XO mice is thus as shown in Fig. 14, the most useful sex-linked markers for this purpose being tabby, *Ta*, and blotchy, *Blo* (L. B. Russell and Saylors,

1962). In one generation XO animals are distinguished from heterozygous XX females by their full expression of tabby, due to the lack of a normal gene from their mother. In the succeeding generation they are distinguished by the lack of the tabby gene which their XO mother carried (Welshons, personal communication).

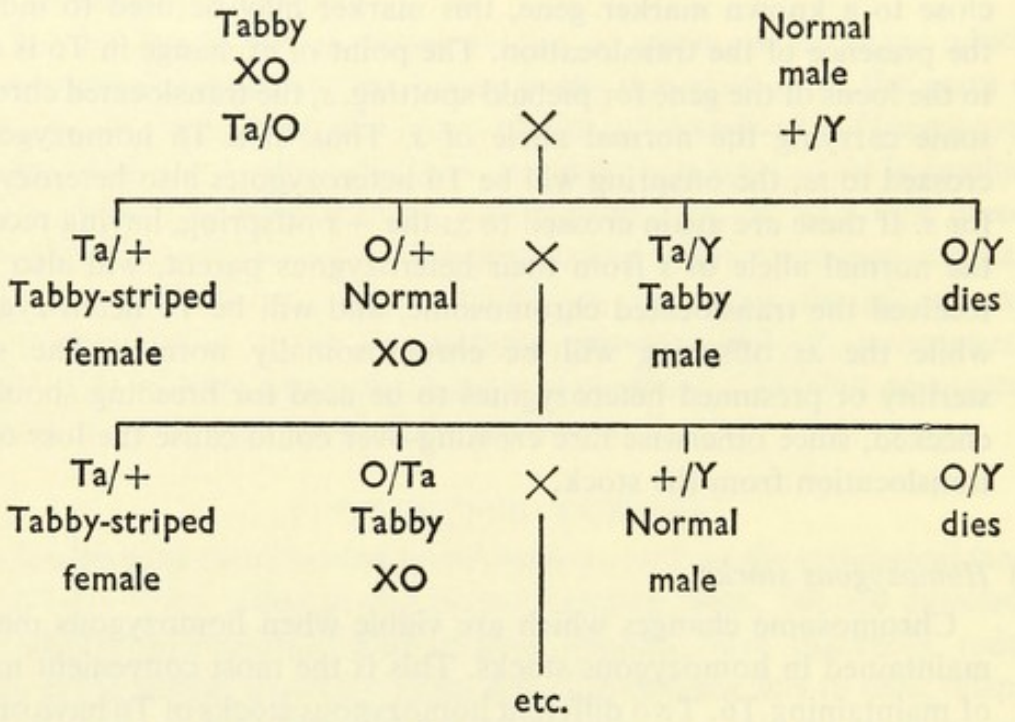


FIG. 14. A technique for maintaining stocks of XO mice.

D. PREPARATION AND MAINTENANCE OF ISOGENIC STRAINS

Although in other paragraphs the preparation of stocks has not been described, it is difficult to consider the maintenance of isogenic stocks without at the same time describing how they are prepared.

1. Isogenic stocks of mutant genes

Mention of some of the methods of preparing and maintaining isogenic stocks of mutants has already been made in the paragraphs on maintaining dominant and recessive genes, but they will be collected together here. These methods depend largely on whether or not the mutant arose in an inbred strain.

a. Mutant arising in an inbred strain. In this case the mutant will from the first be fully isogenic with the non-mutant animals of the strain except at the locus in question. Therefore, the only action needed to maintain an isogenic stock is to keep matings within the strain. As pointed out above, these matings should not be allowed to become homozygous for the mutant but should be backcrosses, e.g., $+p \times pp$ or $Sl+ \times ++$, so that litter-pairs of normal and mutant animals can be obtained. Examples of such stocks include CBA-*p*, which arose by mutation of the gene *p* in strain CBA and is main-

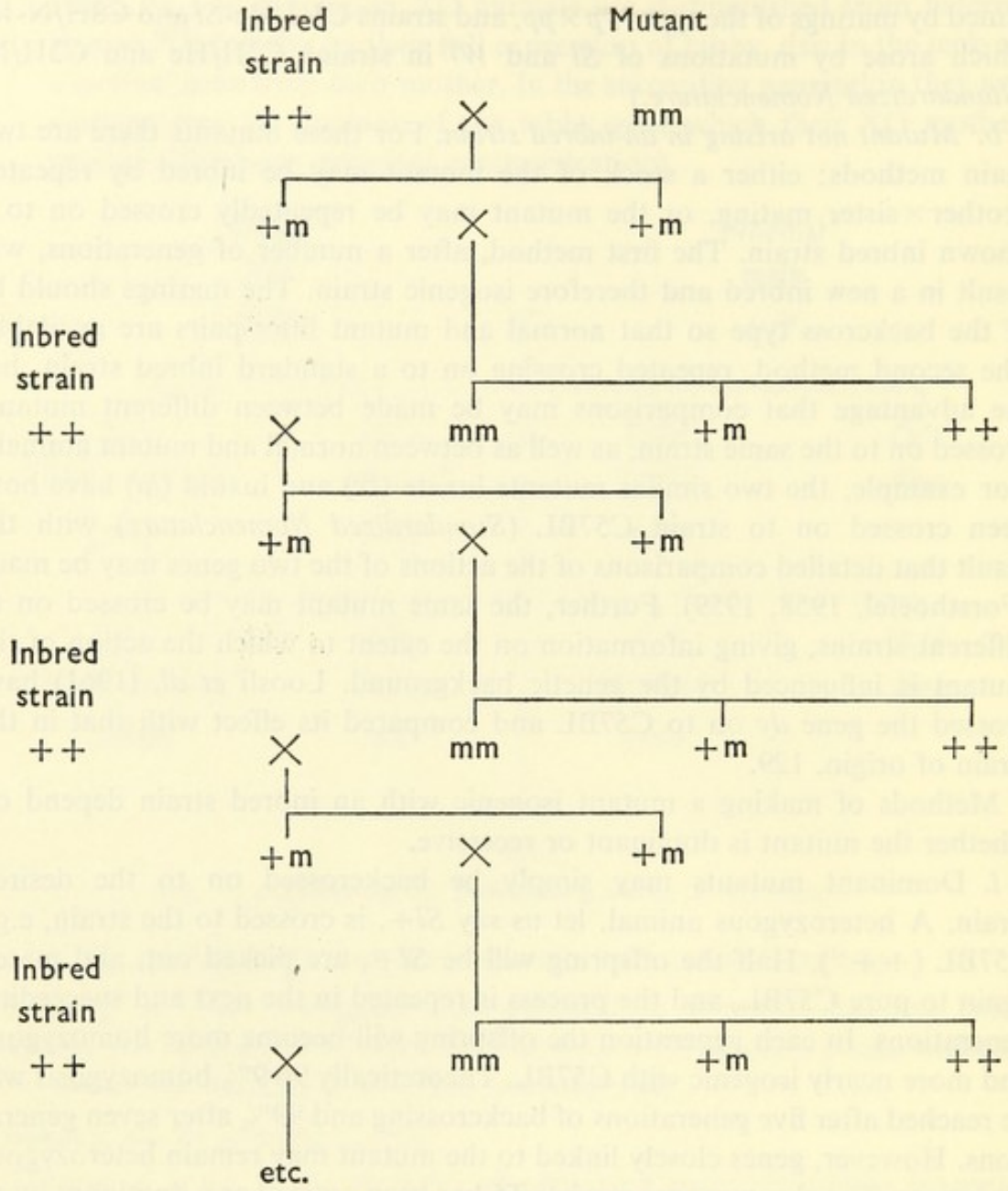
tained by matings of the type $+p \times pp$, and strains C3H/He-*Sl* and C3H/N-W^j which arose by mutations of *Sl* and W^j in strains C3H/He and C3H/N. (*Standardized Nomenclature.*)

b. Mutant not arising in an inbred strain. For these mutants there are two main methods: either a stock of the mutant may be inbred by repeated brother × sister mating, or the mutant may be repeatedly crossed on to a known inbred strain. The first method, after a number of generations, will result in a new inbred and therefore isogenic strain. The matings should be of the backcross type so that normal and mutant litter-pairs are available. The second method, repeated crossing on to a standard inbred strain, has the advantage that comparisons may be made between different mutants crossed on to the same strain, as well as between normal and mutant animals. For example, the two similar mutants luxate (*lx*) and luxoid (*lu*) have both been crossed on to strain C57BL (*Standardized Nomenclature*) with the result that detailed comparisons of the actions of the two genes may be made (Forsthoefel, 1958, 1959). Further, the same mutant may be crossed on to different strains, giving information on the extent to which the action of the mutant is influenced by the genetic background. Loosli *et al.* (1961) have crossed the gene *dy* on to C57BL and compared its effect with that in the strain of origin, 129.

Methods of making a mutant isogenic with an inbred strain depend on whether the mutant is dominant or recessive.

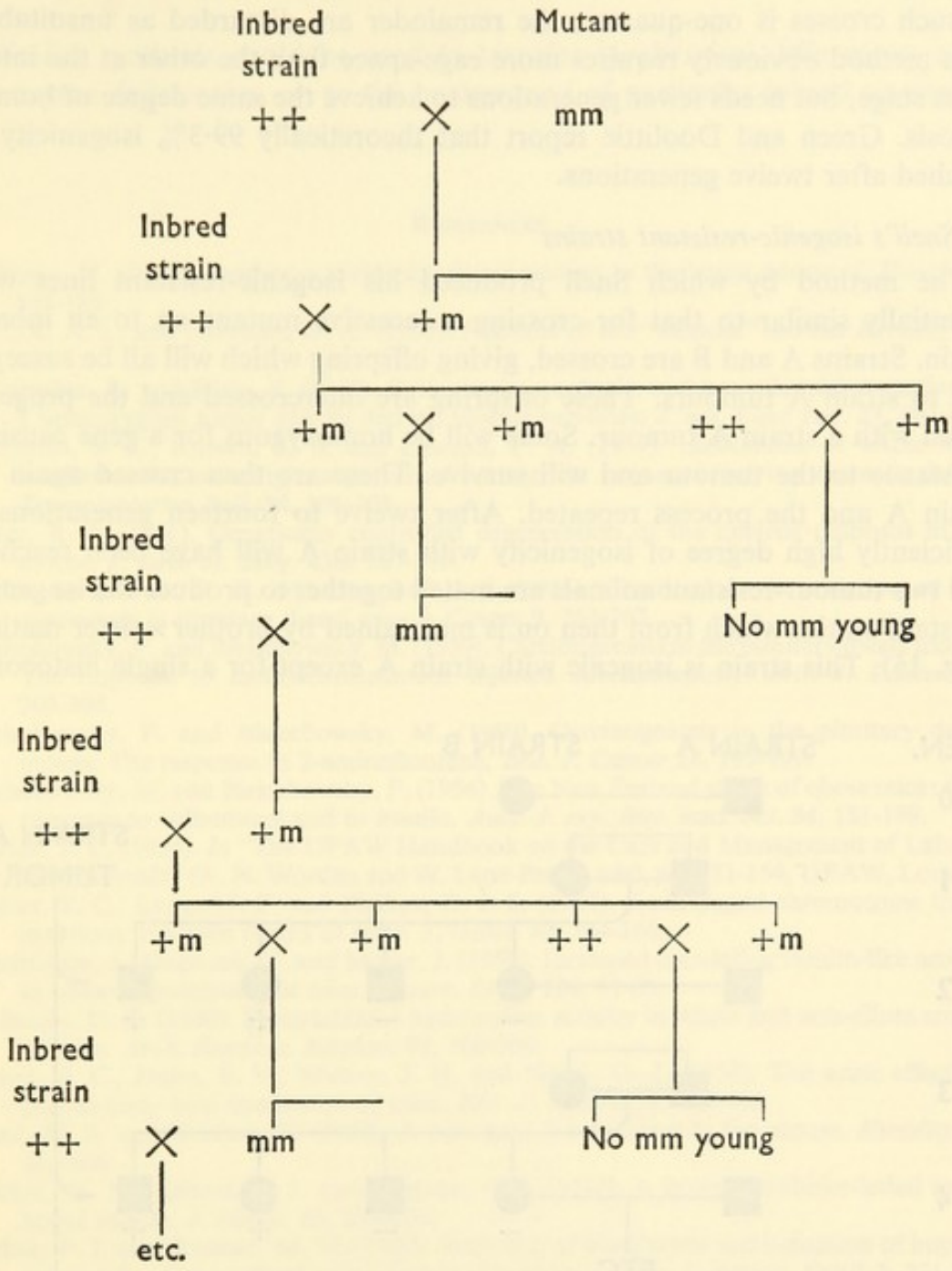
i. Dominant mutants may simply be backcrossed on to the desired strain. A heterozygous animal, let us say *Sl*+, is crossed to the strain, e.g., C57BL ($++^{sl}$). Half the offspring will be *Sl*+, are picked out, and mated again to pure C57BL, and the process is repeated in the next and succeeding generations. In each generation the offspring will become more homozygous and more nearly isogenic with C57BL. Theoretically 96·9% homozygosis will be reached after five generations of backcrossing and 99% after seven generations. However, genes closely linked to the mutant may remain heterozygous for longer. The chromosome marker T6 has been crossed as a dominant on to strain CBA (*Inbred Strains List 2*, Harwell contribution). In this case, since the isogenic strain was needed for immunological work, the immunological criterion of acceptance of skin grafts was used to show at what stage approximate isogenicity was reached. These grafts were first satisfactorily accepted after nine generations of backcrossing. After enough generations to achieve isogenicity the strains may be maintained either by continuous backcrossing on to the inbred strain, or by brother × sister matings within the new strain. In the CBA-*T6* strain, backcrossing was continued up to thirteen generations, and then followed by brother × sister mating. The C57BL-*lu* and C57BL-*lx* strains are continuously backcrossed.

ii. Recessive mutants need a rather more complex method of crossing. The first step is to cross a homozygous mutant animal with the inbred strain.



A. The cross-intercross method.

All the progeny will then be heterozygous for the mutant. If these heterozygotes are again backcrossed to the inbred strain only half the young will carry the mutant, and they will be indistinguishable from those which do not carry it. Therefore, repeated backcrossing would cause the mutant to be lost and crosses to the inbred strain must be interspersed with brother × sister matings to recover homozygous mutant animals. There are various methods of doing this. The first is to make alternate crosses to the inbred strain and intercrosses to recover homozygous mutants, which are then again crossed to the strain (Fig. 15A). The total number of generations needed to approximate isogenicity is of course greater than by successive backcrossing, and it



B. The cross-backcross-intercross method.

FIG. 15. Two ways of crossing a recessive mutant on to an inbred strain.

will take ten generations to achieve 96·9% homozygosity and fourteen to reach 99% (Snell, 1948). Another method, devised by Snell, is referred to by Green and Doolittle (1960) as the cross-backcross-intercross method (Fig. 15B). After the first cross to the inbred strain, the heterozygous offspring are backcrossed again to the strain, and *their* offspring are intercrossed to recover the mutant. Since only some of the backcross offspring will be carriers,

only some intercrosses will throw mutant offspring. The expected proportion of such crosses is one-quarter; the remainder are discarded as unsuitable. This method obviously requires more cage-space than the other at the intercross stage, but needs fewer generations to achieve the same degree of homozygosis. Green and Doolittle report that theoretically 99·3% isogenicity is reached after twelve generations.

2. Snell's isogenic-resistant strains

The method by which Snell produced his isogenic-resistant lines was essentially similar to that for crossing a recessive mutant on to an inbred strain. Strains A and B are crossed, giving offspring which will all be susceptible to strain A tumours. These offspring are intercrossed and the progeny tested with a strain A tumour. Some will be homozygous for a gene causing resistance to the tumour and will survive. These are then crossed again to strain A and the process repeated. After twelve to fourteen generations a sufficiently high degree of isogenicity with strain A will have been reached and two tumour-resistant animals are mated together to produce the isogenic-resistant strain, which from then on is maintained by brother × sister mating (Fig. 16). This strain is isogenic with strain A except for a single histocom-

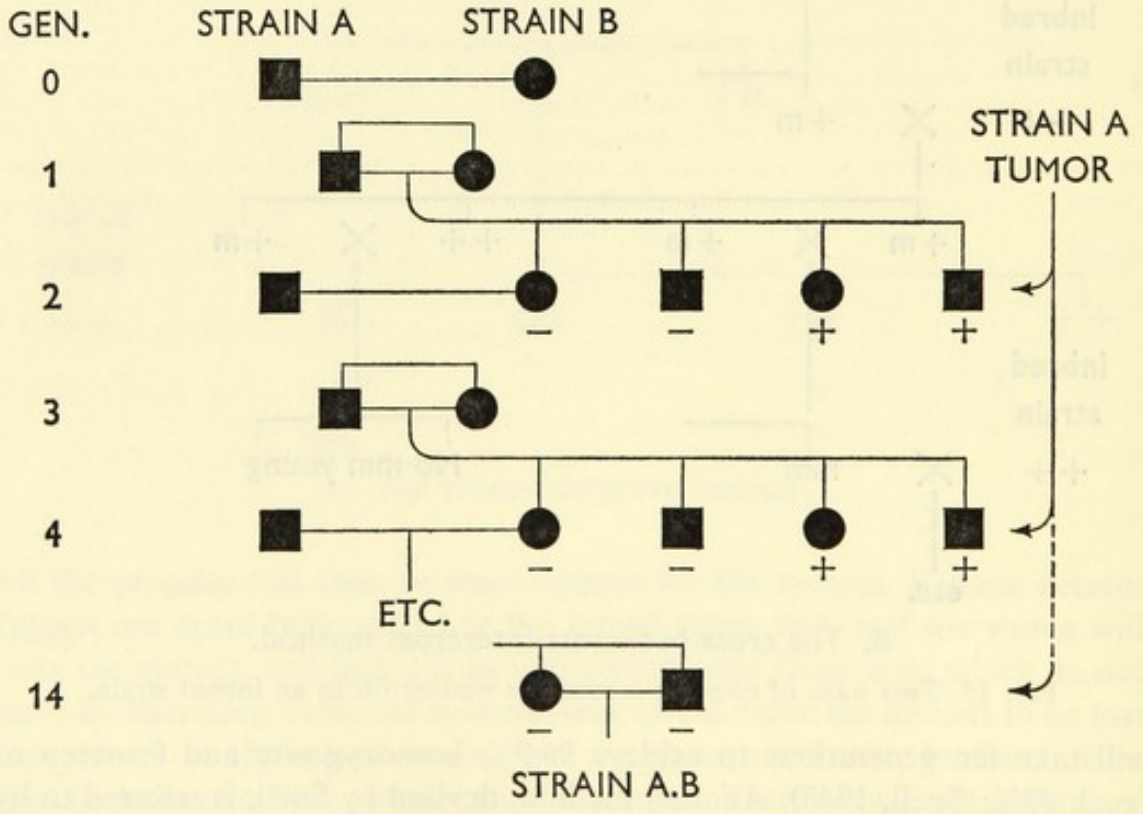


FIG. 16. Method used by Snell for the production of isogenic-resistant lines of mice. The offspring of a cross between strains A and B are intercrossed, and their progeny tested for resistance to a strain A tumour. The survivors, indicated by a minus sign, are mated again to strain A. The process is repeated up to generation 14, when two survivors are mated together to give strain A.B. (From Snell, 1958, by courtesy of the *Journal of the National Cancer Institute*.)

patibility gene from strain B, which is causing resistance to the strain A tumour.

Similar methods could be used to transfer biochemical differences, such as enzyme deficiencies, on to the background of particular inbred strains.

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Chapter 8

Inbreeding and Selection

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The breeding programme of a colony of laboratory animals must be strictly controlled if the animals are to be suited to the needs of the laboratory. Using the correct breeding methods the breeder can alter the characteristics of the stock, thus producing animals better fitted for his purpose. A point of economic importance is the productivity of the colony and this can often be improved by careful revision of the breeding methods.

A colony of laboratory animals is maintained by choosing young individuals which are mated to replace the old breeding stock. The characteristics of the colony are profoundly influenced by the selection of these young animals and by the way they are mated. Matings may be between animals which are closely related, i.e., inbreeding, or between unrelated animals, i.e., outbreeding. Inbreeding and selection are treated separately here, but their effects influence one another in many ways and this is described in the section on selection. Inbreeding and selection are discussed with colonies of mice and rats in mind but, in fact, the principles can be applied to other laboratory animals.

I. INBREEDING

Inbreeding is the mating together of individuals which are related to each other through having one or more ancestors in common. The rate of inbreeding depends on the closeness of the relationship between the mated animals. In many strains of laboratory animals deliberate inbreeding is

practised while in others, the non-inbred or random-bred strains, the mating of closely related animals is avoided so that inbreeding is reduced to a minimum. The consequences of inbreeding are far reaching; the percentage of heterozygosity present in the population is greatly reduced and inbreeding depression is a common occurrence.

A. REDUCTION OF HETEROZYGOSITY

In a wild population of random-breeding animals there is a great deal of heterogeneity and the animals are heterozygous at many loci on the chromosomes. Inbreeding in a population such as this reduces the heterozygosity so that as inbreeding continues the animals become increasingly homozygous and more uniform genetically. The rate of increase of homozygosity is dependent upon the closeness of the relationship between the mated animals.

1. Coefficient of inbreeding

The degree of inbreeding is expressed as the coefficient of inbreeding which, is known as F (Wright, 1921). F measures the percentage reduction in the number of heterozygous loci in the population. For F to be meaningful there must be some base line to which it can be referred. It is usual to take as the base line the population from which the inbred line is derived, or the foundation generation of an experiment. F then represents the percentage reduction in the number of heterozygous loci which has occurred during the course of the inbreeding or the experiment. F increases in value as the inbreeding continues. The rate of increase of F depends on the relationships of the mated animals.

In species where self-fertilization is not possible the highest rate of inbreeding is obtained by brother × sister matings and under this regime F increases by approximately 19% in each generation. Offspring × younger parent matings also result in this high rate of inbreeding in the autosomal genes and give a higher rate, approximately 29%, for sex-linked genes. Less closely related matings result in a slower increase in the coefficient of inbreeding, e.g., F increases by approximately 8% per generation for first cousin matings and by approximately 11% per generation for half-sib matings where one male is mated to his half-sister and the females are half-sisters of each other. Where the females are full sisters the rate of inbreeding is a little higher. These and other values of F for a great variety of mating systems are given by Wright (1933).

As inbreeding continues F increases and its value at any one generation is easily calculated. For example, after one generation of brother × sister mating F is 19%. During the second generation the heterozygosity is reduced by 19% of the amount left after the first generation of inbreeding, i.e., 19% of 81% which is approximately 15%. Therefore, after two generations of

brother × sister matings F is 19% + 15% = 34%. These percentages, which have been used for illustration, are only approximately correct as the rate of inbreeding is not constant during the first few generations of brother × sister matings. As shown by Falconer (1960), the rate of inbreeding for the first four generations is 25%, 17%, 20% and 19%, thereafter settling to a constant value of 19·1%.

The coefficient of inbreeding after n generations can more easily be computed from the following equation:

$$Fn = 1-(1-\Delta F)^n \qquad \text{(Falconer, 1960)}$$

where n is the number of inbreeding generations and ΔF is the rate of inbreeding per generation. For example, after ten generations of brother × sister matings where ΔF is assumed to be 19%, F_{10} may be calculated as follows:

$$\begin{aligned} F_{10} &= 1-(1-0{\cdot}19)^{10} \\ &= 1-(0{\cdot}81)^{10} \\ &= 1-0{\cdot}1216 \\ &= 0{\cdot}8784 = 87{\cdot}84\% \end{aligned}$$

(Note that the value of ΔF cannot be used as a percentage in this equation.) Thus after ten generations of brother × sister matings 87·84% of the heterozygosity present in the original population has been eliminated. (This percentage is only approximately correct because the rate of inbreeding is not constant during the first four generations.) Table I shows the values of F for twenty generations of full-sib matings by which time F closely approaches its maximum value of 100%.

TABLE I

Inbreeding coefficients over the first 20 generations of brother × sister or offspring × younger parent mating (from Falconer, 1960)

Generation	F	Generation	F
0	0·0	11	·908
1	·250	12	·926
2	·375	13	·940
3	·500	14	·951
4	·594	15	·961
5	·672	16	·968
6	·734	17	·974
7	·785	18	·979
8	·826	19	·983
9	·859	20	·986
10	·886		

2. Genetic uniformity of an inbred strain

The accepted definition of an inbred strain is as follows. "A strain shall be regarded as inbred when it has been mated brother × sister (hereafter called

b × s) for twenty or more consecutive generations. Parent × offspring matings may be substituted for b × s matings, provided that in the case of consecutive parent × offspring matings the mating in each case is to the younger of the two parents" (Snell *et al.*, 1960). By definition, therefore, an inbred strain is one which has been closely inbred for twenty generations, by which time the percentage of homozygosity is theoretically very near its maximum. (See Table I.)

It is doubtful whether an inbred strain is ever completely homozygous although, of course, genetic differences between animals of one inbred strain are small compared with differences between inbred strains or between individuals of a non-inbred colony. A small percentage of heterozygosity may be retained in an inbred strain by natural and artificial selection for fertility which favours the heterozygotes at the expense of the more infertile homozygotes. Heterozygosity is also continuously created by spontaneous mutation. For these reasons a strain cannot be regarded as being irrevocably homozygous after twenty generations of brother × sister mating and close inbreeding must be continued for as long as the strain is in existence if maximum homozygosity is to be retained.

3. Genetic variation between sub-lines of inbred strains

Although it is true that homozygosity increases as inbreeding proceeds, it does not follow that a number of inbred lines derived from the same random bred population will be identical; for the lines may become homozygous for different genes. Variation in gene frequency between lines arises by sampling errors which are unavoidable in a small population, and this phenomenon is known as random drift. It follows, therefore, that although inbred lines derived from one foundation population are all more or less homozygous they may quite well have different characteristics, because the same genes have not been fixed in all the lines. Clearly a stock consisting of many lines may exhibit more phenotypic variation than the random-bred population from which the lines were derived.

Separation of an inbred line into sub-lines also results in divergence. Differences arising between sub-lines will be greater if they are separated after only a few generations of inbreeding and much smaller if they are formed after many generations of inbreeding because by this time little genetic variance will remain in the original inbred line. Accumulation of mutations, however, will lead to increasing sub-line divergence in both instances.

Sub-line divergence, estimated by using the mean values of six metric traits of the skeleton, has been demonstrated in two sub-lines of the C57BL/6 inbred strain separated after more than thirty generations of full-sib mating and in two sub-lines of the BALB/c inbred strain separated after seventy-eight generations of full-sib mating (Bailey, 1959). Hamer (1955) found two distinct levels of the enzyme liver B-glucuronidase in different sub-lines of the C3H/Bi

inbred strain and concluded that this was a single gene mutation. Differences in the number of vertebrae in sub-lines of the C3H inbred strain have been demonstrated by McLaren and Michie (1954, 1955). The study of twenty-seven skeletal characters has shown divergence in sub-lines of the C57BL inbred strain separated after a long history of close inbreeding and also in sub-lines of the C57BR inbred strain separated after only nine generations of full-sib mating (Deol *et al.*, 1957; Carpenter *et al.*, 1957). The differences between the C57BR sub-lines are assumed to be due at least to some extent to residual heterozygosity present at the time of separation. In all other cases, however, it is concluded that the differences are mutational in origin.

It is most important, therefore, that sub-lines are clearly designated and it should never be assumed that two sub-lines of an inbred strain are identical. The following rules have been formulated as a guide to the formation of sub-strains. "Any strains separated after eight to nineteen generations of brother × sister inbreeding and maintained thereafter in the same laboratory without intercrossing for a further twelve or more generations shall be regarded as sub-strains. It shall also be considered that sub-strains have been constituted (*a*) if pairs from the parent strain (or sub-strain) are transferred to another investigator, or (*b*) if detectable genetic differences become established" (Snell *et al.*, 1960).

Crossing of sub-lines reduces the coefficient of inbreeding to that of the generation at which the sub-lines were separated. If the inbred strain was highly inbred before the sub-lines were formed, then the loss of inbreeding will not be very great. Crossing of unrelated lines reduces the coefficient of inbreeding to zero.

B. INBREEDING DEPRESSION

Inbreeding depression often results from close inbreeding and constitutes a decline in some or all of the components of biological fitness. Those characters which may be adversely affected include size, growth rate, life span, susceptibility to disease, viability, vigour and all aspects of fertility. Some extensive studies on inbreeding depression in laboratory animals were carried out by Wright (1922) working with guinea-pigs. Such are the consequences of inbreeding that many inbred lines fail to survive and the number in existence today are but a small proportion of those started at various times. Of those surviving, some are difficult to maintain because they exhibit some degree of inbreeding depression. Deriving a new inbred line from a non-inbred colony is best achieved by starting not one but a number of inbred lines from the same foundation stock. After a few generations some lines will be lost because of infertility, and from the remainder the one suffering least from the effects of inbreeding may be maintained, and the others discarded.

Inbreeding depression may be caused by some or all of the following factors.

1. Deleterious recessives

As inbreeding proceeds and the animals become increasingly homozygous, deleterious recessive genes, the effects of which were masked by dominant alleles when in the heterozygous state, now have an overt and often disastrous effect on the nearly homozygous individuals.

2. Homozygosis

Homozygosity *per se* in the absence of deleterious recessive genes may have an adverse effect on the development of the individual and it may be that there is a minimum level of heterozygosity necessary to produce an animal which is sufficiently fit, in the biological sense, for survival (Lerner, 1954).

3. Polygenic unbalance

Large numbers of polygenes, each of which has a very small effect, act together to influence the development of an individual. Natural selection favours those combinations of polygenes which produce biologically fit animals, and in a wild population a balanced polygenic system is achieved. Inbreeding upsets this balance, resulting in poor developmental stability of the individual (Mather, 1955).

Although inbreeding produces animals which are more or less genetically uniform, it does not necessarily lead to phenotypic uniformity. Phenotypic variation can be caused by both genetic and environmental variation. Inbreeding depression may manifest itself by an increase in phenotypic variation which is caused not by genetic differences but by the environment. Highly homozygous animals show incomplete buffering of their development in the face of small environmental adversities. These animals are also vulnerable to the effects of unfavourable combinations of genes and chance mishaps which may occur during development such as unequal division of cells or irregular production or distribution of substances. Lerner (1954) gives an account of the phenotypic variation which may be encountered in inbred animals.

C. MAINTAINING AN INBRED STRAIN

1. Some practical considerations

It is most important that the identity of each animal in the colony is known. One mistake in identity can be a serious set-back to the inbred strain, more particularly as the mistake will probably remain undetected. If there is ever any doubt as to the identity of an animal, that animal should be discarded. It is essential that cages containing animals be adequately labelled, and the animals may be ear-punched or marked in some other way as an extra precaution. If possible, it is also advisable to keep strains of animals which are similar in appearance in different animal rooms.

Permanent single-pair mating is the easiest system to use and, as the male

is never moved from the female, it also reduces the risk of a mistaken identity. This system works well with mice and rats because the male does not interfere with the growing litter. Breeding inbred animals in trios is quite unsatisfactory, for there is often uncertainty about which female is the mother, and mice from two litters of the same age may be mixed.

A brother × sister mating regime is simpler to operate than an offspring × younger parent mating system. With brother × sister matings it is most convenient to use litter-mates, but animals from different litters of the same parents may be mated if need be. This may be necessary where animals in some litters are entirely or predominantly of one sex.

After a large number of generations of inbreeding a less closely related mating, such as a cousin mating, could be resorted to if necessary with little or no harm. A careful record of all such irregular matings should be kept and an adjustment made to the coefficient of inbreeding which reverts to that of the generation of the common ancestors of the mated animals; in the case of a cousin mating this will be the grandparents' generation. However, if such a practice is necessary because of low fertility it is doubtful whether it is worth keeping the strain because, clearly, it will be of little use in providing sufficient animals for experimental purposes. It would be more profitable to discontinue the strain and obtain a new breeding nucleus from a more productive sub-line maintained elsewhere.

Some inbred animals breed well while others are notoriously difficult to maintain. Some only thrive with the addition of food supplements to the diet, and other nervous animals need quiet conditions with only one person handling them. Animals of many inbred strains tend to be temperamental and need careful handling by conscientious animal technicians who are interested in their work. Trustworthy technicians are also necessary to ensure that no mistakes occur in the identity of animals and that accurate records are kept.

2. Sub-lines of inbred strains

It is a readily accepted fact that sub-lines of the same inbred strain maintained in different laboratories may not be identical. It is not so readily accepted that one inbred line maintained in one animal house as one unit may consist of several sub-lines which have been separated for a number of generations. Differences may arise between these sub-lines which will contribute another source of variance in an experiment where animals from the different sub-lines are used indiscriminately. The formation of sub-lines is a result of maintaining the inbred strain by the parallel-line system and can be avoided by using a modification of the single-line system. The latter system is the more troublesome to operate but there is no doubt that it produces more uniform animals and, taking the long-term view, the extra work is worth while.

a. *Parallel-line system.* This is illustrated in Fig. 1a. Five pairs of animals have been taken from the foundation stock to start an inbred line. From the progeny of each pair one male and one female are mated each generation, thus establishing five sub-lines which will become increasingly divergent. Additional sub-lines will be created if more than one pair of animals are mated in each sub-line each generation.

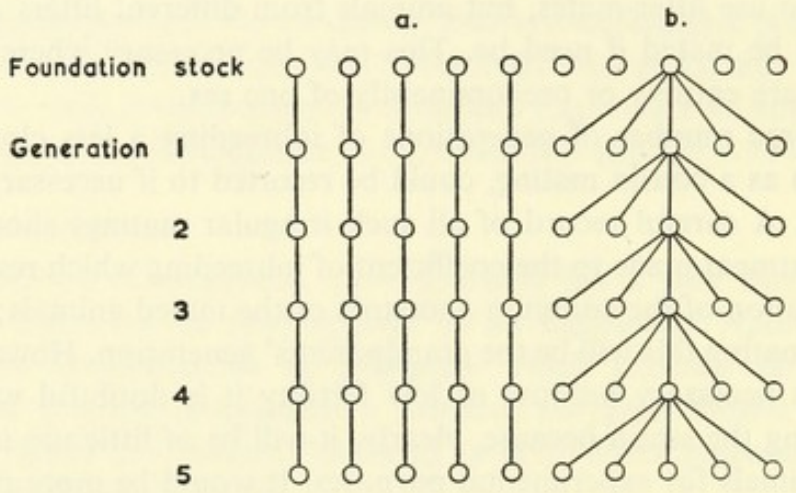

FIG. 1. (*a*) Parallel-line system, (*b*) Single-line system (see text).

b. *Single-line system.* This is illustrated in Fig. 1b. Of the five pairs chosen from the foundation stock, four are discarded and the progeny from only one pair is used to set up five more pairs. If this procedure is repeated each generation, only one line can be traced back to the foundation stock.

It is not necessary to adhere strictly to the single-line system and in practice an inbred strain is usually maintained by a modification of this. In order to produce a useful number of inbred animals and also to give a wide enough field for selection for fertility there must be a number of divergent sub-lines at any one generation. These sub-lines, however, should be continually pruned so that, with the exception of the last few generations, only one line can be traced back to the foundation generation of the strain. To do this it is essential to know which animals constitute the sub-lines and for this purpose a pedigree chart is invaluable.

In this chart, an example of which is shown in Fig. 2, each row represents one generation and each circle represents one breeding pair (the circles may be numbered to identify the breeding pairs). Each new mating is recorded on the chart and a line indicates the parentage of the newly mated animals. With the aid of this chart sub-lines are readily distinguishable and can be kept short. The sub-line chosen to continue the strain should be selected with care. Animals in this sub-line should show any special features characteristic of the strain and should be at least as fertile as animals in the sub-lines which are terminated. In a good breeding strain all sub-lines but one can be terminated

after three generations. In a poor breeding strain they may be allowed to run for four or five generations and, in fact, in a strain with a long history of close inbreeding, they may be allowed to run for about ten generations with reasonable safety, although this will usually be unnecessary.

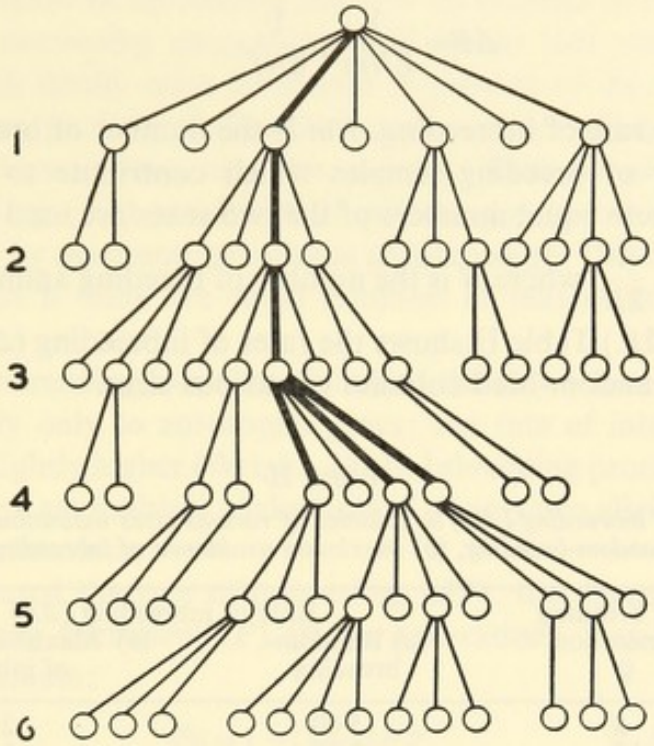

FIG. 2. Pedigree chart of an inbred strain. The thick line represents the single line which can be traced back to the foundation generation. At the fourth generation there are three possible lines; only one of those will be selected to continue the strain.

The aim of inbreeding is to produce uniform animals and it is worth remembering that the retention of sub-lines promotes genetic diversity and the elimination of sub-lines leads to genetic uniformity.

D. MAINTAINING A NON-INBRED STRAIN

In maintaining a non-inbred strain, the main aim is to retain genetic variation by preventing inbreeding. When starting a non-inbred colony it is essential to begin with a large number of breeding animals so that there is sufficient genetic variation present in the foundation generation. The number of breeding animals used each generation to continue the colony must be large enough to avoid mating closely related individuals. However, only if the stock is very large can an increase in the coefficient of inbreeding (F) be avoided over a number of generations.

1. Coefficient of inbreeding

In a closed non-inbred colony there is a gradual increase in the number of loci which are homozygous and, therefore, an increase in the inbreeding

coefficient. The rate of increase depends not upon the total size of the colony but on the effective size, i.e., the number of breeding animals which are used each generation to continue the colony. The rate of inbreeding may be calculated approximately from the following formula:

$$\Delta F = \frac{1}{8Nm} + \frac{1}{8Nf} \qquad \text{(Wright, 1931)}$$

where ΔF is the rate of inbreeding, Nm is the number of breeding males and Nf the number of breeding females which contribute to each successive generation. (Where equal numbers of the two sexes are used for breeding the simpler formula $\frac{1}{2N}$, where N is the number of breeding animals, may be used for calculating ΔF.) Table II shows the rates of inbreeding (ΔF) converted to percentages in random-bred colonies of various sizes.

TABLE II

Rates of inbreeding (ΔF) in colonies of various sizes maintained by (a) random-breeding, (b) maximum avoidance of inbreeding

Numbers of breeding animals/generation ♂	♀	Rate of inbreeding (ΔF) (a) Random-breeding	(b) Maximum avoidance of inbreeding
5	5	5·00	2·50
10	10	2·50	1·25
15	15	1·67	0·83
20	20	1·25	0·62
40	40	0·62	0·31
50	50	0·50	0·25
100	100	0·25	0·12
5	10	3·75	
5	20	3·12	
10	20	1·87	
10	40	1·56	
20	40	0·94	
20	80	0·78	
40	80	0·47	
40	160	0·39	

These formulae for calculating ΔF are valid only if the parents of each generation are mated at random. If mating is not deliberately randomized the rate of inbreeding will be unnecessarily high and the genetic variation will not be randomly distributed throughout the colony. It is worth remembering that the uniform genetic variability of non-inbred animals is just as important as the uniform genetic invariability of inbred animals. Randomization of animals for mating is easily done by using statistical tables of random numbers. Poiley (1960) has published a system to facilitate dealing with large numbers of breeding animals.

Where inbreeding is avoided to a maximum, the rate of inbreeding is halved and the formula for calculating ΔF become $\frac{1}{4N}$, where N is the number of breeding individuals contributing to the next generation (Wright, 1931). Maximum avoidance of inbreeding requires all families to be equally represented in each succeeding generation. This means that one male and one female from each family must be chosen as parents of the next generation. Under this system matings of closely related animals, which may sometimes occur with random mating, must be avoided. Table II gives examples of rates of inbreeding (ΔF) converted to percentages in colonies of different sizes maintained by maximum avoidance of inbreeding. This breeding method can only be used if there are equal numbers of males and females in the breeding colony.

The rates of inbreeding calculated by using the above formulae and shown in Table II apply only to autosomal genes; the rate of inbreeding for sex-linked genes is slightly higher (Wright, 1933). Inbreeding proceeds at a slightly slower rate if there are multiple alleles instead of only two alleles at some of the loci on the chromosomes.

Having calculated the rate of inbreeding (ΔF), the degree of inbreeding, i.e., the inbreeding coefficient (F) after n generations, may be derived from the following formula:

$$Fn = 1-(1-\Delta F)^n \qquad \text{(Falconer, 1960)}$$

Table III shows the degrees of inbreeding attained after 5, 10, 20 and 40 generations in colonies of different sizes for both random mating and maximum avoidance of inbreeding. (Coefficients of inbreeding given as percentages.)

It is clear from Tables II and III that a non-inbred colony maintained by too few breeding animals will have a high rate of inbreeding (ΔF) and after some generations have elapsed the coefficient of inbreeding (F) will reach a dangerously high level. An inbreeding rate of not more than 1-2% per generation is commonly regarded as acceptable and this means maintaining about ten breeding pairs where there is maximum avoidance of inbreeding, and about twelve to twenty pairs or ten to fifteen trios where random mating is used. Taking the long-term view it is clearly better to use more breeding animals if this is possible.

Expansion of the colony to increase the number of breeding animals will reduce the amount of new inbreeding but can do nothing to alter the inbreeding which has already occurred in the previous history of the colony. If the size of the breeding population shows large fluctuations then the inbreeding coefficient is influenced to a large extent by those generations with the smallest numbers of breeding animals. Where there is a difference in the numbers of the two sexes used for breeding, then it is not so much the total number of

breeding animals but the number of the less-frequent sex (usually males) which influences the inbreeding coefficient. This fact will be readily observed by studying Tables II and III.

TABLE III

Coefficients of inbreeding (F) in colonies of various sizes maintained by (a) random-breeding, (b) maximum avoidance of inbreeding for 5, 10, 20 and 40 generations

Numbers of breeding animals/generation		Coefficient of inbreeding (F) (a) Random-breeding Generations				(b) Maximum avoidance of inbreeding Generations			
♂	♀	5	10	20	40	5	10	20	40
5	5	22·62	40·13	64·16	87·15	11·89	22·37	39·74	63·69
10	10	11·89	22·37	39·74	63·69	6·09	11·81	22·23	39·52
15	15	8·08	15·51	28·62	49·04	4·08	7·80	15·36	28·35
20	20	6·09	11·81	22·23	39·52	3·06	6·03	11·69	22·02
40	40	3·06	6·03	11·69	22·02	1·55	3·06	6·03	11·69
50	50	2·48	4·90	9·55	18·19	1·23	2·46	4·85	9·47
100	100	1·23	2·46	4·85	9·47	0·60	1·19	2·37	4·68
5	10	17·40	31·75	53·42	78·30				
5	20	14·65	27·15	46·94	71·84				
10	20	9·01	17·21	31·45	53·01				
10	40	7·55	14·53	26·95	46·64				
20	40	4·61	9·01	17·21	31·45				
20	80	3·84	7·53	14·49	26·89				
40	80	2·35	4·61	9·01	17·21				
40	160	1·94	3·84	7·53	14·49				

2. *Biological fitness of non-inbred animals*

In a non-inbred colony which is sufficiently large to prevent too much inbreeding the animals will retain a large proportion of their heterozygosity and will not be subject to manifestations of inbreeding depression. These colonies are more fertile and viable than inbred strains and, on the whole, are easier to maintain. There will, of course, be some environmentally caused variation between these animals, but as the individuals are less vulnerable than inbred animals to chance fluctuations in the environment, it follows that variation caused by the environment will probably be less in non-inbred than in inbred animals.

E. CHOICE OF BREEDING METHOD

Phenotypic uniformity is desirable for most experimental and assay work because a higher degree of precision is attained as variation is eliminated, and smaller numbers of animals are needed. Inbred animals are often used because of their genetic uniformity but, unfortunately, inadequate buffering of the environment often leads to phenotypic variation so that the inbred animals,

apart from being difficult to breed, may also fall short of the expected phenotypic uniformity. On the other hand, non-inbred animals which are much easier to maintain and have less non-genetic variation are genetically heterogenous and therefore cannot be phenotypically uniform.

Mather (1946) realized the potential usefulness in bio-assay of F_1 animals produced by crossing two inbred lines. These F_1 animals are genetically uniform, which removes one source of variation, and also heterozygous, which should lead to better buffering of the environment and a reduction in the non-genetic variation; thus F_1 animals should have, theoretically, the highest degree of phenotypic uniformity. Although the fertility of the inbred parents of these crosses will not be improved, the heterosis or hybrid vigour of the F_1 animals will improve their viability resulting in higher productivity. Using this system, the inbred strains must still be maintained because animals from these strains are needed continually for crosses. F_1 crosses must not be used for breeding because the F_2 animals they produce will be genetically very diverse owing to recombination of genes and chromosomes which occurs during the formation and fusion of the gametes. It must also be noted that F_1 animals produced from reciprocal crosses may show slight differences due to a maternal influence or the sex chromosomes.

Many workers (e.g., Becker, 1962; Biggers *et al.*, 1958, 1961; Brown, 1961a, 1961b; Chai, 1960; Grüneberg, 1955) have tested the sensitivity and precision of experiments using random-bred, inbred and F_1 mice. There is no general agreement about the type of animal which will give the most satisfactory results in all bio-assay work, and in practice preliminary tests are necessary before a suitable choice can be made for any experiment.

F. PRODUCTION OF INBRED ANIMALS IN LARGE NUMBERS

It is often difficult to produce sufficient experimental animals from an inbred strain without allowing the sub-lines in the strain to be continued for too many generations and abandoning selection of the best animals for breeding. Subcultivation of inbred animals will overcome this difficulty, and the scheme practised by the Laboratory Animals Centre and known as the "traffic-light system" is simple to operate. Animals are taken from the inbred strain and random-bred for three generations only, the progeny from the third generation being used entirely for experimental purposes and not for breeding. Breeding stock is continually drawn from the inbred strain so that the animals retain their identity and three generations of random-breeding are not sufficient to alter the characteristics of the strain. With this scheme in operation large numbers of virtually inbred animals can be produced while the inbred strain itself remains small enough for the exercise of strict health and genetical control.

Lane-Petter (1961) gives details of the Laboratory Animals Centre subcultivation scheme and also discusses the production of laboratory animals

in two stages. The first stage is the inbred strain or primary-type colony, which produces breeding stock for subcultivation in the production units, which constitute the second stage. One such primary-type colony is capable of supplying many production units. These units are not concerned with maintaining the inbred strains as such, so that the establishment of many sub-lines of the same inbred strain is avoided. This means that valid comparisons may be made between experiments performed on animals drawn from any of the production units supplied from the same primary-type colony.

There is no reason why non-inbred animals should not also be produced in two stages and, indeed, many of the advantages of producing inbred animals in this way are also true of non-inbreds.

II. Selection

In a natural population many animals never reach maturity and of those which do survive some leave more offspring than others. The individuals of a heterogeneous population differ from one another genetically and there will always be some which are better adapted to survive and reproduce in their own environment. The ill-adapted animals are less viable and less able to reproduce than the better-adapted animals, and it follows that the greater proportion of the progeny are derived from the latter. In this way natural selection acts upon the genetic variance in a population so that the less fit animals are rejected and the population is maintained primarily by offspring from the more fit animals. Natural selection ensures that the population retains its ability to survive in its own environment. If, for any reason, environmental conditions change, then animals which were ill-adapted to the previous environment may be better adapted to the new environment than the rest of the population. Natural selection will favour these deviants and gradually the characteristics of the population will change as the population becomes better suited to the new conditions. Adaptation to the new conditions can only occur if there is genetic variability in the population, because without the phenotypic differences in animals which it produces, natural selection cannot operate.

Artificial selection is a similar process but the animals selected to continue the colony are those chosen by man and not by natural agencies. In most colonies only a proportion of the animals available for mating are in fact needed. If the choice of animals for mating is not a random one and some animals are preferred to others, then some sort of selection is taking place.

Many selection lines are started to study the effects of selection *per se* while other selection programmes are used merely to alter the mean expression of a character in the colony. Many characters respond to selection, both for increased and for decreased expression, but it is not always possible to predict the effectiveness of any kind of selection. The pattern of response varies; it

may be a slow continuous process lasting over many generations or it may be rapid during the first few generations, after which the selection line reaches a plateau where no further response occurs. Selection of a character in both directions does not always lead to equal response.

Many characters have been subjected to artificial selection; a few examples of these are litter size and lactation in mice (Falconer, 1955), body weight in mice (MacArthur, 1944), blood-pH in mice (Wolfe, 1961) and ovarian response to a standard dose of gonadotrophic hormone in rats (Kyle and Chapman, 1953).

A. RESPONSE TO SELECTION

Selection can only be practised where there are recognizable differences between individuals of a colony. Some of this variation is environmental in origin and as it is not inherited it contributes nothing towards promoting a response to selection. However well-controlled the environment may be, it is impossible to eliminate all non-genetic variation. The variation which is genetic in origin is inherited and contributes a major part to the success of any selection procedure.

Since selection operates on genetic variation it is important that this should be present at the start of the selection experiment. It is very difficult to change the mean expression of a character where there is little variance because selection cannot go beyond the potential of the foundation stock. For this reason it may be impossible to change a character in a strain which has been closely inbred for many generations because the high degree of homozygosity which ensues leaves little genetic variation on which selection can act. It is advisable to begin selection using a heterogeneous stock; inbreeding may be started, if required, as soon as the character of the stock has been altered sufficiently. If inbreeding is started too early, undesirable genes may become fixed and the response to selection will be limited.

The progress which may be expected in a selection experiment depends on the heritability of the character under selection and on the selection differential.

1. Heritability

In each generation of a selection programme the animals are examined and those shown to be superior in the desired character are chosen as parents of the next generation. That part of the superiority which is environmental in origin is lost; the remainder, which is genetic in origin, appears in the progeny and is known as the heritability of the character. Heritability is normally expressed as a percentage and may be calculated from the following equation:

$$H=\frac{R}{S}$$

where H is the heritability, R is the response to selection measured as the average superiority of the progeny over the mean value of the entire parental

generation, and S is the selection differential measured as the average superiority of the parents over the mean value of the entire parental generation.

A theoretical example of selection for increased body weight in mice will serve to illustrate the use of this equation.

Mean value of parental generation	=20 g
Mean value of selected parents	=25 g
Selection differential (i.e., mean superiority of parents)	= 5 g
Mean value of progeny	=22 g
Response to selection (i.e., mean superiority of progeny)	= 2 g

$$H=\frac{R}{S}=\frac{2}{5}=0{\cdot}4=40\%$$

Estimates of heritabilities of different characters show a wide range and cannot generally be predicted in advance of a selection experiment. Complex characters such as fertility and viability usually have low heritabilities. Heritability of a single character is not always constant, and may vary according to the strain, the environment and the method and direction of selection.

Clearly the less environmental variation present the greater will be the heritability of the character. It is therefore advisable to have strict control over all aspects of husbandry to ensure that environmental variation is reduced to a minimum.

2. Selection Differential

The average superiority of the parents over the mean value of their entire generation is known as the selection differential. Clearly this bears some relation to the proportion of animals chosen as parents. The number of animals selected for breeding is determined by the size of the colony required and is normally fixed. The more animals there are available for selection, however, the smaller will be the proportion of those actually chosen and the greater will be the selection differential.

The selection differential based on the percentage of animals chosen for breeding is measured in standard deviations and is known as the intensity of selection. These figures can be calculated from statistical tables of deviations of ranked data (e.g., Table XX of Fisher and Yates, 1948) and are given by Falconer (1960). Table IV gives some examples of these.

TABLE IV

Selection differentials in standard deviations based on percentage of animals chosen for breeding

Per cent chosen	Selection differential
90	0·20
50	0·80
20	1·40
10	1·75
5	2·06

To convert the selection differential from standard deviations to absolute units (e.g., grams, millimetres) the standard deviation is multiplied by the standard deviation of the character under selection. The selection differential, therefore, depends on the proportion of animals selected, i.e., the intensity of selection, and on the variability of the character. Fig. 3 (from Falconer, 1960) illustrates this point.

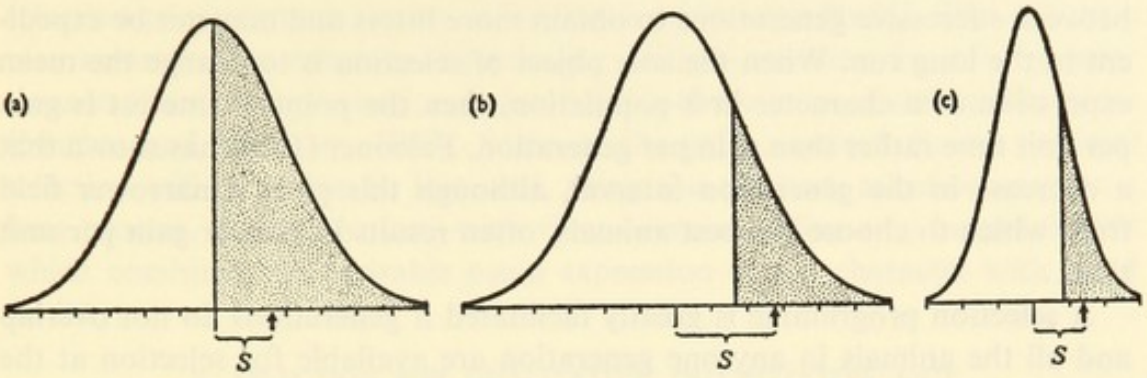

FIG. 3. Diagrams to show how the selection differential (S) depends on the proportion of the population selected, and on the variability of the character. (From Falconer, "Introduction to Quantitative Genetics", Oliver and Boyd, 1960; reproduced by kind permission of the author and publishers.)

The character under selection in each graph in Fig. 3 is assumed to have a normal distribution; the axes are marked in hypothetical units of measurement. Stippled areas represent the percentage of animals selected for breeding, and an arrow marks the mean value of each selected group. S is the selection differential in absolute units.

Graph a. 50% of the population is selected so that the selection differential in standard deviations is 0·80 (see Table IV). The standard deviation of the character under selection is 2 units. Therefore, the selection differential in absolute units (S) is 1·60 units.

Graph b. 20% of the population is selected so that the selection differential in standard deviations is 1·40. The standard deviation of the character under selection is 2 units. Therefore, the selection differential in absolute units (S) is 2·80 units.

Graph c. 20% of the population is selected so that the selection differential in standard deviations is 1·40. The standard deviation of the character under selection is 1 unit. Therefore the selection differential in absolute units (S) is 1·40 units.

Clearly the selection differential (S) increases with the intensity of selection (cf. graphs *a* and *b*) and also with the variance (cf. graphs *b* and *c*).

The rate of progress to be expected in a selection experiment is dependent upon two factors as shown by the following equation:

$$G = H \times S$$

where G is the gain per generation, H is the heritability of the character and S

is the selection differential in absolute units. Thus, the greater the heritability and selection differential, the greater will be the response to selection. In other words, response is promoted by high genetic variance, low environmental variance and high selection intensity. If the colony size is to remain constant then the number of animals chosen for breeding is fixed, but the number of animals from which the choice is made may be increased to give a higher selection intensity. However, this usually means increasing the interval between successive generations to obtain more litters and may not be expedient in the long run. When the sole object of selection is to change the mean expression of a character in a population, then the point of interest is gain per unit time rather than gain per generation. Falconer (1957) has shown that a decrease in the generation interval, although this gives a narrower field from which to choose the best animals, often results in greater gain per unit time.

A selection programme is greatly facilitated if generations do not overlap and all the animals in any one generation are available for selection at the same time. This ideal is not always practicable when working with laboratory animals. A simple way of dealing with the problem of overlapping generations is to choose the best from those animals which are available for mating at any one time regardless of their generation. Usually the selected animals will be from the later generations because they have a longer selection history than animals in the earlier generations and should be superior to them.

3. Factors limiting response to selection

In many experiments a plateau is reached where the line ceases to respond to selection. This limiting of response is caused largely by lack of genetic variance or low fertility.

a. *Lack of genetic variance.* Selection will be ineffective in a line which lacks genetic variance. Some characters may be controlled by relatively few loci and selection produces an initial, rapid response and an early plateau as genetic variance is soon exhausted. If selection for the same character has been applied already in the history of the strain, most of the genes for the character may have become fixed, and little response can be expected. Lines which reach a plateau because of lack of genetic variance are then stable when selection is discontinued. A line which ceases to respond to selection can be tested by applying reverse selection (i.e., selecting in the opposite direction) and if this is successful then clearly some genetic variance remains and some other cause for the plateau must be sought.

b. *Low fertility.* In a great many selection lines there is a correlated response of decreasing fertility, and the extreme phenotypes may be inviable or sterile (Sunder, 1960). Where this occurs a line may reach a plateau despite the fact that genetic variance is still present (Dinsley and Thoday, 1961). Lines reaching a plateau because of correlated changes in fertility have an

unstable equilibrium and if selection is relaxed natural selection acts to improve fertility and the lines revert at least part of the way back to the mean of the original population.

The mean expression of a character is altered by choosing those combinations of genes which give the most extreme phenotypes. In so doing the natural balance of these genes is destroyed and, as a result, the linked fertility genes also become unbalanced and fertility is impaired. The usefulness of any selection programme is limited if fertility is adversely affected. This difficulty may sometimes be overcome by relaxing artificial selection for a few generations to allow natural selection to reconstitute the balance of the fertility genes (Mather and Harrison, 1949). Some of the gain acquired during artificial selection will of course be lost, and, using this method, selection may be a long process. Taking the long-term view, however, a line may be constituted which combines the desirable mean expression of the character with good fertility.

B. SELECTION FOR QUALITATIVE, QUANTITATIVE AND THRESHOLD CHARACTERS

1. Qualitative characters

Qualitative characters show discontinuous variation and for this reason are often called all-or-none characters. They are controlled by major genes each of which has a large and obvious effect, so that segregation of the genes results in recognizable discontinuity between individuals. These are the classic Mendelian genes, and their inheritance can be traced by Mendelian methods. The most familiar major genes are those controlling coat colour, and their effects are described in Chap. 6. Many other examples of major genes in the mouse are included in Chap. 7. Grüneberg (1952, 1956) has catalogued and described all the main mutant genes in the mouse which were known at the times of publication.

Selection of major genes is quick and easy; two or three generations of breeding will usually fix or eliminate a major gene and any subsequent mutation is easily discarded. Where a dominant gene is under selection, progeny testing of individuals is necessary to determine which are homozygous. Selection may be more difficult if the effect of a major gene is masked by other gene effects or if the character is anatomical or physiological and thus more tedious to classify.

2. Quantitative characters

Quantitative characters, also known as metric characters, show continuous variation. They are controlled by a large number of polygenes, each of which usually has an effect too small for segregation to give recognizable differences between individuals; it is very difficult, therefore, to study individual polygenic effects. Metric characters usually have a normal distribution with the

peak of the curve coinciding with the mean expression of the character. Some examples of these characters are body size and weight, growth rate, litter size and lactation. A character may in fact be influenced by both major genes and polygenes, e.g., body weight in mice.

As continuous characters show differences not of quality but of quantity, selection is much more tedious, involving measuring and not simply counting individuals. Selection may also be a lengthy process because the effects of single polygenes cannot be recognized, and response to selection depends on choosing groups of genes which together enhance or diminish the character as required. These genes are brought together only gradually by recombination during the formation of the gametes, and in each generation those combinations of genes producing the most extreme phenotypes are selected.

If selection is discontinued because the objective has been achieved, the line may remain stable at the new level, but in some cases, especially where fertility is low, the line may gradually revert towards the mean of the original population. In such cases inbreeding may be necessary to fix the character. Where inbreeding is undesirable, then continued or intermittent selection will be necessary to maintain the new expression of the character. Mutations of polygenes are not easily recognizable but, if a number of them are allowed to accumulate, their combined effect may be sufficient to change the character of the population; they can be eliminated only by reselection in the line.

During selection of one character correlated responses may occur in other characters which are not directly influenced by the selection programme. These correlated changes may be due to one of the following: pleiotropy of genes, i.e., one gene controlling more than one character; linkage of genes on the chromosomes; the influence one character has over another, e.g., body weight in mice has some control over ovulation rate (Falconer and Roberts, 1960). Mention has already been made of the change in fertility which often occurs during selection and this is probably one of the correlations explained by linkage. On occasions it may be possible to change a character by selecting for a correlated trait. This method of selection is useful if a character does not respond to direct selection or is difficult to measure. Direct selection for sex ratio in mice was found to be ineffective (Falconer, 1954), but direct selection for blood-pH in the mouse produced a correlated change in sex ratio (Weir, 1953).

Simultaneous selection for two or more characters is not usually very effective. This practice leads to less rigid selection of all characters because some compromise must be made when choosing animals for breeding, and the animals so chosen will rarely be superior in all traits. It is only expedient to select for two characters simultaneously when one of these is fertility. Clearly, the usefulness of any selection programme is limited if fertility is impaired and, for this reason, when choosing breeding animals it may be necessary to compromise between fertility and the other trait under selection. Simultaneous

selection for more than one character may be necessary to improve the economic value of livestock, and a selection index as described by Lerner (1950) is the best way of doing this.

3. Threshold characters

Some polygenically controlled characters are subject to a threshold mechanism which operates at, for example, a certain concentration of enzyme, thus giving an all-or-none reaction. Such characters are inherited exactly like continuously varying traits but, because of the threshold, their inheritance appears to be discontinuous since only two classes of individual are recognizable. Threshold characters are said to show quasi-continuous variation, and examples include susceptibility to disease or drugs where the animal either lives or dies and there are no intermediate reactions.

Selection of a character such as drug susceptibility requires extra care in planning the selection programme. By chance, the proportion of susceptible animals may be the same as the proportion required for breeding. It may be, however, that there are more susceptible animals than are needed for breeding, and it is impossible to select further within this group since there are no obvious differences between individuals. If the incidence of drug susceptibility is 50%, and only 10% of the animals are needed for breeding, then choosing these at random from the 50% will give the same selection differential as breeding from the entire 50%. This reduced selection differential will retard the response to selection and is clearly undesirable. As it is the threshold which determines the selection differential, its position must be altered so that the percentage of susceptible animals is similar to the percentage required for breeding. This may be done by adjusting the dosage of the drug. Measuring the time between administration of the drug and subsequent death may also be a useful way of distinguishing between susceptible individuals.

Selection for disease or drug resistance is easily accomplished because those animals left alive after the test are then used for breeding. When selecting for disease or drug susceptibility, however, all animals must be allowed to breed before they are tested, and only offspring from susceptible animals subsequently used to maintain the line. Alternatively, animals closely related to those which are susceptible may be used for breeding although they themselves have not been tested (see Family Selection, p. 256).

C. METHODS OF SELECTION

In any selection experiment individuals are measured or tested and the best are chosen for breeding. Close relatives of individuals, however, can often provide additional information which can usefully be fed into the selection programme.

1. Individual selection

In this method of selection each individual is judged entirely on its own merits without reference to any other animal. The animals with the most extreme phenotypes are chosen and mated together. This is the simplest procedure to follow and is usually the most effective. It may, however, lead to some inbreeding if the selected animals are derived from only a small proportion of the families available.

2. Family selection

Using this method the family is selected as a unit and its performance is judged on the mean value of members of the family. Selection in an inbred strain must be of this type. If close inbreeding is to be avoided, however, a number of families must be selected and some of the offspring from these used for breeding. In order that selection can operate, more families must be raised than are needed for future breeding, and a great deal of space is usually necessary for this method of selection.

Family selection is necessary where the character to be selected cannot be measured on the individual or cannot be measured while the animal is alive, as is the case in drug or disease susceptibility and with anatomical traits. Female fertility characters clearly cannot be estimated in males, so the selection of males must be based on information obtained from sisters or other closely related females. In these experiments selection is based on the average value of members of the family, excluding those individuals which are finally chosen as parents.

3. Within family selection

In this method the best individual is chosen from each family and the mean value of the family itself is ignored. This method is very useful in maintaining a non-inbred colony because each family can be represented in the next generation by choosing one male and one female as parents. The selected animals are deliberately mated at random, and inbreeding is avoided to a maximum. This method is economical of space as the number of families raised is equal to the number of pairs of animals required for future breeding.

D. SELECTION FOR FERTILITY

Natural selection exerts some effect on biological fitness; animals which are sterile or seriously handicapped in some way leave no offspring, and animals less fit than the rest of the population leave few offspring. When breeding laboratory animals, however, it is essential that none of the offspring of these less robust and less fertile animals is used to continue the strain, and only offspring from the best animals are chosen for future breeding. If natural selection is supplemented by artificial selection in this way, the intensity of

selection for biological fitness of the colony is greater than if natural selection alone were allowed to operate.

When choosing animals for mating, those which are sick, in poor condition and underweight will obviously be rejected. Fertility is a complex of characters, some of which are very difficult to measure, and can only be estimated by allowing the animal to breed. The simplest measure of fertility is productivity, i.e., the number of offspring weaned per unit time. An index of productivity "Q" (Lane-Petter *et al.*, 1959) calculated from the number of animals weaned and the intervals between successive litters is a useful way of measuring productivity and a simple way of comparing the performance of different breeding pairs so that the most prolific can be selected. This index of productivity is comprehensive, covering fertility of both parents, mating ability, internal environment of the mother, development of the foetuses, health, lactation and mothering instinct of the mother and viability of the offspring to weaning age. Selection for productivity will also lead to the rejection of animals which are debilitated because of an inapparent infection.

A colony of animals is of little use if fertility is low and for this reason selection for fertility should be included in the selection programme. It is essential to practise this selection in the early stages of the foundation of a new colony, for it may be impossible to regain an acceptable level of productivity once this has been lost. Many inbred strains are difficult to breed and there is little doubt that selection for productivity helps to keep some of them in existence. Both natural and artificial selection for productivity in an inbred strain will probably retain some heterozygosity, and a balance may be reached between reduction of heterozygosity by inbreeding and maintenance of heterozygosity by selection (Hayman and Mather, 1953). This residual heterozygosity will probably not affect the usefulness of the strain and, indeed, may be essential for its survival.

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Chapter 9

Matching the Animal with the Experiment

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I. The Animal to Hand and Advances in Biology During the Past 200 Years

The "respectable antiquity" of the use of animals for biological studies, gradually evolving in pattern until "laboratory familiars" (Lane-Petter, 1961) usurp first attentions, should not deter laboratory workers from seeking among the many species the best tool for their job.

In earlier times the animal to hand was all important and history relates its indispensability in toxicology, in nutrition studies, in microbiology and immunology, in psychology and in medicine and surgery. The various uses of animals in these disciplines from the early nineteenth century clearly show the scientist's attitude towards living creatures.

A. As a substitute for man in toxicology

Sir Robert Christison's *Treatise on Poisons*, published in 1829, tells "how powerful an instrument of research that science has found in the effects of

poison on the animal body". Again, writing of the investigator, Christison says "physiology . . . instructs him how to determine the value of the evidence from the accidental effects of suspected substances on domestic animals, as well as to apply express experiments on animals to settle by analogy doubtful questions relating to the operation of poisons on man". He also states, "but an important objection has been made to both, namely, that what is poison to man is not always poison to the lower animals, and that, on the other hand some of the lower animals are poisoned by substances not hurtful to man". His species of choice were the cat and the dog.

1. The stimulation of classical physiology and anatomy

It was the effects of poison on animals that stimulated physiologists to investigate the nervous system. Magendie by 1822 had distinguished motor and sensory nerves using rabbits, asses, monkeys and puppies. A series of papers, delivered by Sir Charles Bell before the Royal Society, on the nervous system of the human body was published in 1824, and by 1850 Marshall Hall had studied the reflex action in the frog. At this time the pattern for the use of animals in physiology became clear, the choice of animal always subordinating to the system to be studied. Sir Charles Singer in his book *A History of Biology* states that "physiology alone is, however, of its nature incapable of presenting any picture of the mode of action of the organisms as a whole, though modern doctrines of the workings of the nervous systems have given some explanation of many forms of animal behaviour".

2. The effect on pharmacology

In his justification for the use of animals, Christison expressed a sentiment all pharmacologists should welcome. He writes that "in particular cases topics may arise which have not hitherto been investigated, and which it may be necessary to determine by the experiments alluded to. Experiments on animals instituted for such purposes by a skilful toxicologist are not liable to any important objection."

Here we have introduced the idea of the pharmacological screening programmes that now elucidate the action and toxicity of all new compounds prepared by the pharmaceutical industry.

It was soon discovered that the dose of poison cannot be extrapolated between the species. Charret in 1827 stated that 2 drachms of opium are required to kill a middle-sized dog while 26 grains have killed a man. Alcohol on the other hand acts more powerfully on dogs than on man. It was evident that many species were necessary for this work and at the First International Pharmacological Congress in Stockholm in 1961 the subject was brought up to date by Burns of Burroughs Wellcome Company, Tuckahoe.

B. IN THE PROBLEMS OF ADEQUATE NUTRITION

The study of nutrition emerged as a science as the result of the advances made in organic chemistry in the nineteenth century that provided the chemical knowledge of the structure of fats and sugar. Its practical applications were directed to two main subjects.

1. Farming problems

The first experimental station for research in agriculture was founded at Rothamsted, in Hertfordshire, England, in 1843. It was the outcome of John Bennet Lawes' interest in medicinal plants which his inheritance of his father's Rothamsted estates enabled him to pursue. Here Lawes and J. H. Gilbert discovered that protein from cereal and legume seeds differed in nutritive value. A comprehensive account of this early work on feeding farm animals is given by McCollum (1956) in *A History of Nutrition*, and shows clearly that all these experiments were conducted with economics in mind.

2. Human necessities

All animals are susceptible to hunger, and work on nutrients with human needs in mind soon used many species. Rats, now one of the commonest experimental animals in nutrition work, were first employed by W. S. Savoury in 1863. The modern scientific method, however, had been introduced in 1791 by Dr. George Fordyce, F.R.S., who, working with hen canaries to determine the necessity for calcium in diet, was the first experimenter to use animals with a control.

It was following the questionings of J. B. A. Dumas on the effects of food on the infants of Paris, during the German siege (1870-1871) concerning the adequacy of the diet containing protein, carbohydrates, fat and salts, that the study of essential metabolites gradually took shape (Dumas, 1871). In this fascinating advance the names of Hopkins, Eijkman, Funk, Holst, McCollum Chick and Mellanby became familiar, and pigeons, guinea-pigs, rats, rabbits, dogs, pigs and human beings all played their part (McCollum, 1956; Chick, 1928; Mellanby, 1921). The confusion that arose during this time over the requirements of nutrients for differing species must be avoided in current work.

C. AT THE BEGINNING OF MICROBIOLOGY AND IMMUNOLOGY

We owe the birth of microbiology and immunology to Louis Pasteur.

1. Germs as the origin of disease

In 1861 Pasteur exploded the theory of spontaneous generation and summarized his work in *Les Microbes organisés; leur rôle dans la fermentation, la putréfaction et la contagion.* The animal to hand had supplied the answers to silk-worm disease, to fowl cholera and to anthrax in sheep, and the

inspiration from this work led to the isolation of all the common bacterial pathogens during the next thirty years.

2. Vaccines, sera, chemicals and antibiotics for treatment

Disease calls for treatment and Pasteur's attenuated bacteria were the first specific remedies to be applied. The observation of Von Behring followed in 1890 that something in the blood serum of guinea-pigs recovering from diptheria would protect other guinea-pigs from the disease. So the era of specific antitoxin treatment was entered and the horse became the factory for production.

As the biological specifics were introduced, so the idea of specific treatment with chemicals took shape. Successful chemotherapy against protozoa was practised in 1906 when mice infected with trypanosomes were cured by the arsenical atoxyl. Ehrlich and his team, the discoverers in 1909 of Salvarsan (606), the chemotherapeutic cure for syphilis, acknowledged their debt to animals by defining the chemotherapeutic index as the ratio between the lowest possible toxicity for animal host and the greatest parasiticidal action. Until the 'thirties, when prontosil was introduced, there was little advance in chemotherapy but the output of sulphonamides that followed prontosil vastly increased the need for screening programmes, with mice as the animals of choice—the animal to hand was making way for a laboratory familiar.

Nature's source of chemotherapeutics, the antibiotics, was found with the discovery of pencillin by Fleming in 1928 (Fleming, 1929). The very successful *in vivo* application to laboratory animals was followed by outstanding success in man, stimulating a search for other and better antibiotics that has been unabated.

D. IN ENDOCRINOLOGY

With the increased physiological investigation at the end of the nineteenth century, the attribute of each endocrine gland was gradually determined, but it was not until 1901 that adrenaline, the first hormone to be isolated in crystalline form, was obtained from the suprarenal glands of mammals by Takamine and by Aldrich. The thyroid hormone isolated in 1914 by Kendall was identified by Harington and Barger, and subsequently synthesized by them in 1927. But perhaps the most spectacular isolation, because of its clinical impact, was that of insulin in 1921 by Banting and Best from the pancreas of dogs (Banting and Best, 1922). Its assay in mice still necessitates the use of large numbers of laboratory mice every year. Other crystalline hormones were isolated between 1930 and 1940, in particular those of the gonads and of the pituitary glands, and several are still assayed in animals.

E. AS A TOOL FOR THE PSYCHOLOGIST

In considering the early scientific employment of animals, the psychologist cannot be omitted. From Boring's book *A History of Experimental Psychology*

we learn that in 1872 Darwin discussed the "expressions of emotions in man and animals", while Spalding in 1873 published *Instinct with Original Observations on Young Animals*. The first book of comparative psychology, *Animal Intelligence*, written by Romanes in 1882, contains a mass of information on animal behaviour. This was followed in 1890-91 by Lloyd Morgan's *Animal Life and Intelligence*, in 1894 by his *Introduction to Comparative Psychology*, and in 1900 by his *Animal Behaviour*. The *Introduction to Comparative Psychology* gives the methodological principles and canon of interpretation of experiments that were midway between nature study and laboratory. By 1898 Thorndike had introduced puzzle boxes for cats, dogs and chicks, and in 1901 W. S. Small used a maze with rats as the experimental animals. But it was not until 1907, when Cole published an article "Concerning the Intelligence of Raccoons", that the powers of imitation were studied, an attribute that more recently has been followed in apes and monkeys.

As for social psychology, Sir John Lubbock in 1882 published *Ants, Bees and Wasps—Social Insects*, but in the most fascinating work on this subject apes have been used (Zuckerman, 1932).

F. IN MEDICINE AND SURGERY

Animals used in medicine are essentially the same as those necessary in the general biological sciences. In surgery, however, experimental work is generally confined to the larger animals whose organs and vascular system are more suitable in size for operations that could be applied to man. Perhaps the most rewarding surgery in recent years, pioneered in dogs, resulted from the invention of the heart-lung machine by Gibbon in 1937. In England experimental open-heart surgery made possible by this machine was done on greyhounds by Melrose *et al.* (1953) before any application was made to man. So the animal to hand in Christison's day has become a laboratory animal of choice today, which compels us to consider that every experimental animal should be matched to its task.

II. Animal Characteristics and Research Projects

Each species of animal has many characteristics—anatomical, physiological specific pattern of metabolism, typical behaviour, breeding habits and genetic status. The empirical reactions of each species to foreign substances varies, and the degree to which all the characteristics of a species are documented depends on its popularity as a laboratory animal. A closer inspection of these differential attributes will show how important they are in the choice of animal for any particular research project.

A. ANIMALS WITH CHARACTERISTICS RESEMBLING THOSE IN MAN

These are in general the larger mammals and more especially other primates, so it is probably wise to begin this series of animals with the primate order.

1. Monkeys and apes

Similar characteristics to those in man are found in the anatomy, psychology, physiology and disease susceptibility of both the monkey and the ape. Recently it has been suggested that the pygmy marmoset could well take a very useful place with these as an experimental animal (Sobel *et al.*, 1960) and at least one pharmaceutical firm is taking steps to learn how to breed it in captivity.

Inhalation experiments, as for example in the chronic toxicity of anaesthetics (Robertson, *et al.*, 1947), are often designed to use monkeys because of the anatomical likeness of their lungs to those of man. An extensive study of the physical characteristics of the chest and lung, and the work of breathing, has been made recently in several mammalian species by Crosfill and Widdicombe (1961).

Behaviour studies too have favoured the use of monkeys and apes. Some have been notorious, such as the study of bringing up a chimpanzee with a child and noting the relative superiorities of each (Kellogg and Kellogg, 1933), or more recently and of an academic nature, the study of the behaviour of the chacma baboon, *Papio ursinus* (Bolwig, 1959). Reproduction and growth of the primates closely resembles that in man and makes studies of the gestation period and training in chimpanzees (Peacock and Rogers, 1959) and of the induction of multiple ovulation in rhesus monkeys, *Macaca mulatta* (van Wagenen and Simpson, 1957), doubly valuable. It is interesting that in the study of human pituitary dwarfism, monkey pituitary growth hormone is found to stimulate humans (Beck *et al.*, 1957; Knobil and Greep, 1959), while ox hormone, although able to stimulate growth in dwarf rats, fails to do this in humans (Kinsell, 1955).

In blood-group studies primates have their special place as being the only animals with red cells that cross agglutinate with humans. The review by Gilmour (1961) of blood groups of animals other than man gives useful information about many species.

In susceptibility to specific disease, particularly in the course of the infection and paralysing effects of polio virus, the monkey was for a time man's only experimental animal (Amoss, 1928).

2. Cattle and goats

The economic interests of the farmer in dairying has made the study of lactation a necessity, and the knowledge obtained has been gainfully used in studying the same function in humans. In England, research workers at the National Institute for Research in Dairying have made many important contributions (Folley and French, 1949, 1950; Popják *et al.*, 1952). In this work the samples of milk used were taken from sheep, goats or cows, but biochemical work was done on the tissue of the mammary gland from rats,

rabbits and guinea-pigs, the latter being the animal of choice if easy homogenization is required (Folley, private communication).

Sheep have been used for some of the most profitable studies on pre-natal life, notably those directed by Sir Joseph Barcroft and described in *Researches in Pre-Natal Life* (1946).

3. Pigs

Intestinal resemblance to man is the reason accepted for using pigs as experimental animals for evaluating dietary effects on physiological systems. Platt and Stewart (1960) recently studied the central nervous system of pigs on low protein diet.

4. Dogs

Because of a small stomach and short intestinal tract resembling the structures of man, and because a dog can be conditioned to carrying a stomach cannula, it is the animal of choice for studies in digestion. The classical work of Pavlov on conditioned reflexes in the dog, associating hunger and signals, is world famous (Pavlov, 1910). In modern work on gastric secretion the same animal is employed (Johnston and Code, 1960).

Its ability to learn also allows a trained dog to be used unanaesthetized in renal studies (see Perlès, Colas and Blayo, 1960).

B. ANIMALS WITH UNIQUE FEATURES OF ANATOMY AND PHYSIOLOGY

The nature of the physiologist's discipline demands that he should explore unique features of anatomy for their effect on normal functions, and in the sister discipline of pharmacology the action of drugs on individual organs is frequently required. In microbiology, too, some unique features of anatomy have made possible the study of the bacterial flora of isolated organs.

1. Cattle and Sheep

In the ruminant with its multiple stomach, where cannulae are easily inserted, slow digestion aids the study of nutrition (Paloheim and Makela, 1959). The bacterial flora of the rumen has been of particular interest and extensively investigated by Blackburn and Hobson (1960a, b and c).

2. Dogs

A discrete pancreas allowing ready resection enabled all the classical work on diabetes to be done on the dog, which is still the animal of choice in pancreatic investigation (Stewart *et al.*, 1958). Moreover, the size and accessibility of its vascular system, coupled with its ability to learn, makes the dog invaluable for experiments on circulation that need unanaesthetized animals (Armstrong *et al.*, 1961; Wien, 1961). In the article by Wien quoted here, reference is made to other interesting techniques for the measurement or modification of blood pressure in rats, rabbits, monkeys and dogs.

3. Cats

In Great Britain the use of the cat as a laboratory animals causes more contention than that of any other animal. In 1954 D'Amour and Blood published a *Manual for Laboratory Work in Mammalian Physiology* describing the use of rats for many classical physiological procedures where cats are usually employed. This was favourably reviewed by Folley (1955). There are experiments, however, where if for no other reason the size of the rat as compared with the cat precludes its use.

It can safely be said that in the study of nerve centres in the brain, cats are essential. Brain maps for cats are available and, provided female cats ofknown age from one breeding centre are used (as these have more constant head size), the wastage in animals is small (Abrahams *et al.*, 1960). Rabbit brain maps have recently become available (Monnier and Gangloff, 1961), but for certain studies, particularly those requiring stable blood pressure, these animals are contraindicated.

In every type of research when stable blood pressure is required, workers use cats (Drăskoci *et al.*, 1960). Their vein walls are much stronger than those of the rabbit, the other common laboratory animal of comparable size, and this has been suggested as the reason for the stability.

One other unique feature of the cat is that its nictitating membrane is more highly developed than in any other laboratory animal, so that membrane contractions may be used to record intracranial stimulation (Rothballer and Sharpless, 1961; Boura *et al.*, 1961).

4. Guinea-pigs

The sensitive cochlea of the guinea-pig has allowed of its use in hearing experiments (Legouix and Moulonguet, 1957; Deatherage *et al.*, 1959). Further, its docile nature has made it of use in experiments on oxygen consumption, for which it is more suitable than rats or mice (Harvey, 1958). It is said to be four times as resistant as the mouse and twice as resistant as the rat to hypoxia.

5. Hamster

The hamster has only of comparatively recent years become a common laboratory animal and its unusual cheek pouches are now used as sites for tissue culture. A number of human cancers have been successfully heterografted to the hamster cheek pouch (Chute *et al.*, 1952; Pierce *et al.*, 1957).

6. Rats and mice

These animals are particularly suitable for the study of the physiology of the liver. In 1947 Himsworth described the rat as a suitable animal for a partial hepatectomy, because when 60-70% of the liver tissue is removed the organ regenerates almost completely in the course of a week. For this reason

rats were used by Benacerraf *et al.* in 1957 when measuring blood liver flow. Further, it has been found when using rats that 90% of the phagocytic activity in the circulatory system is attributable to the Kupffer cells of the liver (Halpern *et al.*, 1957).

For experiments affecting the reticulo-endothelial system in which Kupffer cells are specially involved, albino TO mice are eminently suitable (Nicol and Bilbey, 1958).

An extensive study of the architecture of the circulatory systems of the liver in the human, rat and other mammals has been made by Bilbey and Rappaport and was reported to the 7th International Congress of Anatomists in 1960. An abstract of this work is to be found in the *Anatomical Record*, and further details may be obtained from Dr. Bilbey, Sub-Dean of the Medical School, King's College, Strand, London.

7. Frogs

An interesting recent use to which frogs have been put is that of determining the retinal toxicity of drugs for the chemotherapy of schistosoma infection. As retinal damage is a symptom of the disease it is important that the retinal toxicity of any drug used for its treatment is known. It was found by Goodwin *et al.* (1957) that light bleaches rhodopsin from frog retina in one hour and that it is resynthesized in one hour in the dark. Certain drugs prevent its resynthesis in proportion to the dose given, and the effect can be used to assay their toxicity.

8. Marine animals: the octopus and electric fish

Already in its natural state the octopus drills holes in the shells of molluscs and opens mussels (Pilson and Taylor, 1961) and large nerve axons have made it a suitable animal for the study of the physiology of learning (Boycott and Young, 1956). In electric fish, the biophysics of adapted electric organs are readily investigated (Bennett *et al.*, 1961).

C. ANIMALS WITH SPECIAL FEATURES OF METABOLISM

Here we cross the border between physiology and biochemistry and an extremely useful section with tables of metabolic activity appertaining to the tissues of a wide spectrum of animals is contained in the *Biochemists Handbook*, edited by Long (1961). But the knowledge of the unusual metabolism of particular substances in the whole animal has to be obtained by word of mouth or from the literature. The impressions received by Dr. Wien of May and Baker Limited from J. J. Burns at the International Pharmacological Congress mentioned in the introductory section are given below.

"Burns made two points: not only were there species differences in relation to the metabolism of different species, but also there was different metabolism of a drug from one patient to another patient. He quoted the following

figures for phenylbutazone (the figures shown are what he described as the biological half-life, presumably the time for the metabolism of the drug): man, 72; monkey, 18; dog, 6; rabbit, 3; rat, 6; guinea-pig, 5; horse, 6. In monkeys the metabolism of this drug was slower than in the dog, but faster than in man. For trommexan the biological half-life in man was 2; rabbit, 2; dog, 18. The type of metabolism was different in rabbit and man. In rabbit, it was metabolized to a trommexan acid, and in man to hydroxyethyl coumarate. With hexobarbitone metabolism occurred very quickly in the mouse, but lasted many hours in the dog. Apart from obvious genetic differences which may account for these differences in metabolism, another explanation put forward was that metabolic variations may be explained in terms of the activity of drug-metabolizing enzymes in liver microsomes, and that the activity of these liver-microsomal systems may be different in different species."

Another useful source for information is the review on *Methods used in determining Chronic Toxicity* by Barnes and Denz (1954). The list of common laboratory animals below and their respective peculiarities has been gleaned from various sources.

1. Dogs

Rigorous investigation of the metabolism of the sulphonamides in a number of animals has revealed that dogs differ from other mammals (Wien, private communication).

2. Cats

Because of their ability to produce methaemoglobin it has been found that cats are the most suitable animals to use for the toxicity testing of compounds such as acetanilide and acetophentidine (Lester, 1943). Dogs and rats produce some methaemoglobin but are not as suitable as cats, while monkeys and rabbits produce none.

Cats are also very sensitive to all phenols, and this idiosyncrasy extends to other members of the cat family such as lions and tigers. A dose of phenothiazine per kilogram body weight that is tolerated by sheep, cattle and horses will kill a lion or tiger (Poulsen, private communication).

3. Rabbits

An interesting method for the bio-assay of progestin in rabbits has arisen from the production in immature animals, primed with oestrone, of specific anhydrase activity (Ogawa and Pincus, 1961). The production of a similar effect in mice has not been found possible. The convenience in the rabbit of injecting one ear, and obtaining a blood sample from the other, is unique to this laboratory species and of practical importance in this particular assay.

4. Guinea-pigs

One of the most surprising idiosyncrasies in metabolism is that of the guinea-pig for penicillin. Miescher and Böhm in 1947 stated that penicillin is one hundred to one thousand times more toxic to the guinea-pig than to the mouse.

5. Chickens

These are readily available experimental animals that are often overlooked. They can be obtained germ-free or used in their natural state, and they have several metabolic peculiarities. They more closely resemble man in their responses to the demyelinating effects of organo-phosphorus compounds than any other animal (Smith *et al.*, 1932; Barnes and Denz, 1953). Their high metabolic rate makes them particularly suitable for the assay of the Vitamin B complex, especially Vitamin B_{12} (Coates *et al.*, 1956). They are used for work with Vitamin D because, as in man, a clear-cut deficiency is obtained on a diet without a grossly altered calcium to phosphorus ratio (Coates and Holdsworth, 1961).

6. Ducks

These other domestic fowls that invade the laboratory have been used for the measurement of lens opacities in dinitrophenol cataract (Robbins, 1944), and for the detection of aflatoxin in ground nuts (Blount *et al.*, 1963).

D. ANIMALS WITH ABILITY TO LEARN

The ability to learn is shared by many animals, and although perhaps the monkey, dog and rat are those species that come to mind first, others have been used. In Section II A (page 266) indications were given for the choice of the monkey or the dog, in Section II B (p. 269) for the octopus, and they will be given in Section II G (p. 274) for the rat.

E. ANIMALS WITH USEFUL BREEDING HABITS AND HIGH PRODUCTIVITY

It is small mammals and insects that are usually associated with high productivity and in this section mice and fruit flies begin to show their worth.

1. Mice

Where studies on breeding in different environmental temperatures have been made mice are first favourites. Much of the work has been done by Barnett and his collaborators (1959, 1960a, b, c) and by Harrison (1958).

Mice have been used for the population study of a free-roaming colony in a battery of cages, which could be arranged with access between sections of varying size. The mice were allowed freedom to roam as and where they would, and the increase in population was allowed to proceed until living conditions depressed its rate (Godfrey, private communication).

[1] In a germ-free strain of guinea-pigs recently tested by Formal *et al.* (1963), sensitivity to penicillin was absent and could not be induced by contaminating the animals with *Escherichia coli.*

It is hardly necessary to say that mice have been and are being used for genetic studies, in a school in the U.S.A. led by G. D. Snell at the Jackson Memorial Laboratories, Bar Harbor, at the Institute of Animal Genetics in Edinburgh, and elsewhere. For certain work inbred strains are not essential and selection in random-bred strains can yield interesting results (Falconer, 1959).

2. Drosophila

As an insect *Drosophila* meets the need for rapid breeding in that several generations can be studied in a comparatively short time. Further, it has only four pairs of chromosomes and a convenient set of giant chromosomes in the salivary gland. Hence the large volume of genetic research for which this animal is responsible (Altenburg, 1958; Dinsley and Thoday, 1961).

F. ANIMALS USED FOR THEIR INBRED STATE

One of the first workers to bring to the fore the usefulness of the genetic state of an animal in research was Elizabeth S. Russell. In a paper given before the Animal Care Panel in the U.S.A. in 1953 she made a masterly survey of the advantages to be gained in using not only the right species but the most suitable strain of that species (Russell, 1955). The laboratory animals most commonly used in their inbred state are guinea-pigs, rats and mice (Jay 1963).

1. Inbred guinea-pigs

Guinea-pigs were the first species to be highly inbred (see Chap. 10) and homozygosis is still an advantage for work in allergy (Stone, 1961).

2. Inbred rats

For similar reasons workers have stated the necessity for using inbred rats for allergy studies (Koprowski *et al.*, 1960). Recently, in a Wistar strain of rat, Harris and West (1961) found a proportion of animals resistant to anaphylactoid reaction produced by dextran. Sprague-Dawley and hooded Lister rats, also non-inbred, all reacted rapidly. The characteristics of this strain specificity are being followed by the inbreeding of selected resistant rats from the original Wistar strain.

In the cancer field it has recently been found that the newer benzpyrene-induced tumour takes more successfully in an inbred colony of hooded rats (C. Smith, private communication). This is in contrast to the Walker sarcoma that may be induced readily in rats maintained as a closed colony.

Other selective breeding found useful in rats is that applied by Smirk and Hall (1958) when developing a colony of hypertensive animals.

3. Inbred mice

The use of inbred mice is becoming widespread and is already much commoner than in any other vertebrate species. In the fruit-fly, *Drosophila*, the

possibility of a multitude of genetically separate strains has been greatly exploited for the study of inheritance, but the ready availability of inbred strains of mice enables the use of this species to be gainfully extended to many disciplines. Of particular interest to man is the study of cancer in inbred mice. Mühlbock (1958) states "that it cannot be emphasized too strongly how much the use of inbred strains has contributed to cancer research". Spontaneous tumours differ from strain to strain, genetic factors control the degree of susceptibility to chemical carcinogens, and transplantation studies can be made in no other living medium with such facility.

To the radiobiologist inbred strains are indispensable when studying genetic adaption, longevity and carcinogenicity (Maisin, 1958) and in certain aspects of tissue immunity and immunogenetics the same may be said (Billingham *et al.*, 1954; Brent and Medawar, 1961). On the other hand some immunological problems make it necessary to use all species (Medawar, 1958).

Studies in pure genetics with inbred mice have led to the isolation of pathological mutants. Some of these have been produced spontaneously as the "Dreher" mutant (Fischer, 1956), while others occur during back-cross mating (Deol, 1956). Many are mentioned in *An Annotated Catalogue of the Mutant Genes of the House Mouse* by Grüneberg (1956).

The differential characteristics revealed by their varied responses has promoted a study of the relation between genetic pattern and the specific responses of inbred strains and their F_1 hybrids. This is being pursued by Chai and his team at Bar Harbor, U.S.A., Grüneberg and his team at University College, London, and Lane-Petter and his team at Carshalton, Surrey. Although it is unlikely that it will be possible from this study to postulate either the anatomical or physiological reactions of strains in new situations, an accumulation of such knowledge should prepare an experimenter for what may happen, and help him to choose suitable strains for preliminary investigations.

G. ANIMALS WITH USES WHERE DOCUMENTATION COUNTS

1. Uniform strains of mouse including inbred strains

A uniform strain of mouse may be defined as one propagated by the selection with random numbers of breeding pairs from a closed colony of not less than ten pairs per generation. An inbred strain is made by brother × sister mating of the progeny of the more productive lines of a strain, these reducing by selection to a single line and maintaining it with standard husbandry. Such strains are invaluable in control work and in large-scale research.

In screening programmes mice are used almost exclusively whether for the chemotherapy of bacteria, of viruses or of parasites. Mice are not necessarily as susceptible as other animals, but their size and availability serve to make them the animal of choice. In the search for a cancer chemotherapeutic agent

inbred strains are used, and in the U.S.A. mouse production for this purpose is on an ambitious scale and adequately described by Jay (1960). In all screening work records of strain susceptibility and reaction are built up so that eventually a strain of mouse becomes valuable for what is known about it.

For empirical reactions to foreign substances, and experiments that can come under the heading of toxicity testing, the mouse is a very satisfactory animal. It is for this work that the documentation of the differential characteristics of strain is so essential. Chai in 1960 reviewed the response in mice to hormones; Brown in 1959 described the response in uniform mouse strains to histamine acid phosphate, and to pentobarbitone sodium, and reviewed work on other barbitone compounds. More recently, extensive work on the responses of inbred, hybrid and random-bred mice to insulin and to pentobarbitone sodium has been published by Brown (1961a and b). This work is in accord with the statement by Gaddum in 1953 that there is little or no direct evidence that genetic homogeneity of itself increases the uniformity of responses to drugs. It is evident, however, that both in pharmacological screening tests and in assay, advantage could be taken of specific strain differences.

The popularity of mice for behaviour studies is increasing and a selected bibliography was published by Joan Staats in 1958 for inbred mice. An interesting paper on social dominance in inbred strains was published by Lindzey *et al.* in 1961, and a paper by Mordkoff and Fuller in 1959 indicates that the behaviour of strains other than inbred repays investigation.

2. Uniform strains of rats

This species although not used in such large numbers as the mouse is perhaps more exclusively required for what is known about it than any other (Broadhurst, 1963).

Behaviour studies in rats have been made ever since their introduction to the laboratory and recently interest has been shown in sex and strain differences (Woolley *et al.*, 1961). Another current study is that of the reaction to infantile handling (Bovard, 1958; Denenberg and Karas, 1959).

In the routine screening of the toxicity of new compounds, both acute and chronic, the rat is often used. It is cheap, fertile, hardy and has a short lifespan. Many pharmaceutical firms breed their own animals and have past records of normal and treated animals with which to compare current tests. The existence of strain differences has been known for many years (Dieke and Richter, 1945) and introduces its own difficulties in assessing differences in results obtained in parallel tests in several laboratories.

In reproductive physiology the knowledge acquired in husbandry is invaluable, and rats have been used for the study of the oestrous cycle, mating behaviour, and lactation (Bruce, 1961; Doyle and Clegg, 1961).

For similar reasons nutrition studies have often been made in rats, and as

long ago as 1938 strain differences in Vitamin B_1 requirement were noted by Light and Gracas. Recent work has included amino-acid imbalance, trace metal studies, and selection and growth in relation to planes of nutrition (Rechcigl *et al.*, 1959; Mills, 1960; and Kidwell *et al.*, 1960).

3. Cats

The recording of brain maps for cats and their evident value has already been mentioned on page 268.

H. ANIMALS USED FOR THEIR DISEASE SUSCEPTIBILITY

When animals are used for studies of disease susceptibility, it may be of diseases to which the particular animals are subject, or of human diseases. The first problem is essentially that of the veterinarian and the microbiologist. As an example of this, it is possible to find in the animal house of a pharmaceutical laboratory turkeys being investigated for their toxic reactions to compounds produced for the chemotherapeutic treatment of black-head, because they are the only susceptible species. For similar reasons it is conceivable that any of the smaller farm animals could be found as temporary laboratory inhabitants. Mention should also be made of the tropical diseases leischmaniasis and schistomsomiasis for which hamsters have been found so valuable. No other small rodent is as susceptible to direct infection with the *Schistosoma* cercaria, and the high yield of eggs produced is used in mice as a source of infection against which to assess the value of new chemotherapeutic drugs.

Fungal diseases too, have attracted more attention of late and techniques for the *in vivo* screening of anti-fungal agents against some of the more usual infections are described by Taylor and D'Arcy (1961).

I. UNUSUAL SPECIES AND THEIR USES

Research workers are necessarily fastidious in their choice of tools and the mention of a few of the more unusual laboratory animals that have been used recently seems appropriate. A number of others, particularly those of the lower orders, have been described by Schmidt-Nielsen (1961).

1. Multimammate mouse

A description of the multimammate mouse (*Rattus* (*Mastomys*) *natalensis* Smith) and its various attributes was published by Davis and Oettlé in 1958. It is an African wild rodent of which a high proportion develop spontaneous adenocarcinoma of the glandular stomach. It is also highly susceptible to schistosoma.

2. Hibernating animals: bats, hedgehogs and ground squirrels

As recently as 1961 the effect of hibernation on homograft tolerance was studied in bats by Mohos, who also gives explicit information on their

laboratory maintenance. Eliassen and Leivestad (1961) investigated the sodium and potassium in the muscles of hedgehogs and bats, and some laboratory data on hedgehogs hibernating and non-hibernating have been collected by Biörck *et al.* (1956). The ground squirrel (*Citellus lateralis*) is the third unusual laboratory animal in which hibernation is studied. (Pengelley and Fisher, 1957).

3. Chinese hamster

In cancer studies where chromosome numbers are important the Chinese hamster (*Cricetulus griseus*) is often the animal of choice (Yergonian and Gagnon, 1958).

4. Steppe lemming

The introduction in Russia of the steppe lemming (*Lagurus lagurus* Pall) to the laboratory is described by Pogosianz in 1958 in her paper at the ICLA Symposium. It is a non-hibernating mammal useful for skin cancer and heterotransplantation of tumours (Pogosianz *et al.*, 1960).

5. Japanese quail

Particulars of the husbandry of the Japanese quail (*Coturnix coturnix japonica*) were given in 1959 by Padgett and Ivey. It reaches maturity rapidly and is suitable for genetic studies. A recent paper on chicken-quail hybrids was published by Wilcox and Clark (1961).

III. Animals Essential to Biological Routine

In numbers of animals used, but not in variety, routine far exceeds research, particularly when large-scale assay is considered. Work is always proceeding to reduce this number, for whether in hospital finance or in that of biological production this cost must be as low as possible. An attempt will be made here to cite those animals now employed for particular tasks, but mention will also be made of some which have been or could be used for similar purposes either in routine or research.

A. Animals used in diagnosis

1. Mice, rabbits, toads and frogs for pregnancy tests

It is many years since Aschheim and Zondek devised the pregnancy test in mice that is now rarely used as a routine. The same may be said for the Friedman test in rabbits. Most hospitals in the United Kingdom now use British male toads, injecting pregnant urine to produce spermatogenesis. Particulars of these tests and others are given by Stitt *et al.* (1948), Bell (1952) and Loraine (1958).

2. Guinea-pigs in the diagnosis of bacterial and viral infection

In vivo confirmation of human bacterial diseases is limited almost entirely to those caused by *Mycobacterium tuberculosis*, *Brucella abortus* and *B. melitensis* and *Corynebacterium diphtheriae*, and for all of these specific autopsy findings are present in the guinea-pig. With the decrease in the incidence of both tuberculosis and diphtheria, the number of animals used in diagnosis has necessarily greatly diminished. Viral diseases for which the guinea-pig has been used in diagnosis are Q fever and lymphocytic choriomeningitis.

3. Rabbits as antiserum producers

These are used almost exclusively in the preparation of diagnostic sera against bacteria, viruses and blood groups.

B. ANIMALS AND THE DEMANDS OF THE THERAPEUTIC SUBSTANCES ACT (U.K.) AND OF THE *British Pharmacopoeia*

The phenomenal increase in the importance of the laboratory animal that took place in the 1930s can be traced almost entirely to the Therapeutic Substances Act of 1925 and to the Permanent Commission of Biological Standards appointed by the Health Committee of the League of Nations about 1928. As a result of this Commission, biological assays were included for the first time in the appendix of the *British Pharmacopoeia* 1932 edition. The use of animals for assay accounts for a large proportion of the Home Office returns, and although, as the methods of preparing chemically pure therapeutic substances expand, more and more of these can be assayed by chemical means, a hard core of biological estimations remains. Animals used in this way will be dealt with in three sections.

1. Animals for the assay of (substances commonly known as) vaccines, sera, toxins and antitoxins and antigens

Vaccines for human use against pertussis and poliomyelitis require for their preparation the use of large numbers of animals. For the assay of pertussis vaccine, mice that are sensitive to intracerebral infection with *Bordetella pertussis* are required, while for the assay of poliomyelitis vaccine, monkeys are used. These can be of several species, but in the safety test some preference is shown for the cynomolgus monkey, *Macaca irus*. Other species that have been employed in both the preparation and testing of the vaccine are the rhesus, vervet and patas monkeys. In the preparation of the vaccine it is important that the kidneys employed for tissue culture should be free from wild virus.

Vaccinia virus, the third common vaccine used for human beings, is produced in calves or sheep and is sometimes passaged through rabbits. As it

can now be freeze-dried for storage, its production entails fewer animals than heretofore.

The cultures for vaccines against distemper virus in dogs are grown and tested in eggs, although the final vaccine is still assayed in either ferrets or dogs. Leptospirosis vaccines, also prepared for use in dogs, are assayed, *Leptospira icterohaemorrhagiae* in guinea-pigs and *Leptospira canicola* in hamsters.

The animals used in the assay of sera, toxins and antitoxins are most usually rabbits, guinea-pigs and mice. Antibacterial sera and toxins are assayed in animals to which the bacteria or toxins that they antagonize are susceptible, so that diphtheria antitoxin is assayed in guinea-pigs, tetanus antitoxin in mice, and scarlet fever antitoxin in rabbits. Since the advent of chemotherapy and antibiotics and the production of prophylactic agents, these sera have been in small demand.

For the assay of antigens other than vaccines used for the prevention of human diseases, of which diphtheria and tetanus are the most common, guinea-pigs are used. Particulars of the official assays for all classes of compounds in this section are to be found in the *British Pharmacopoeia* (1958).

2. Animals for the assay of insulin and other hormones

It is certain that of all the hormone assays insulin estimation uses the most animals and receives the most criticism. Particulars are given in the *British Pharmacopoeia* (1958) of the estimation using mice. The strain of mouse is important as some non-inbred colonies are much more sensitive to the hormone than others, and there are wide differences in the sensitivities of inbred mice (Brown, 1961a). For long-acting insulins, such as protamine zinc insulin, mice and rabbits are used, and for the test for prolongation of insulin effect by these substances, either rabbits or guinea-pigs.

The official estimation of chorionic gonadotrophin uses female rats, that of corticotrophin and vasopressin, male rats, while in the assay of oxytocin chickens are favoured. These animals are not necessarily the only ones in which the above hormones may be assayed, for mammotrophic agent has been estimated in mice of the A2G strain by Young (1957). It is interesting that thirty-seven mouse strains were investigated by Young and of these only two were found to react sufficiently to be used as test animals.

Many hormones may be estimated in one of a choice of several animals, and there are times when the least usual animal may be the most satisfactory one to employ. Thyroid hormone may be estimated by the very sensitive method using metamorphosis in the tadpole (D'Angelo *et al.*, 1942; D'Angelo *et al.*, 1951), androgen by comb growth in baby chicks rather than in cockerels (Munson and Sheps, 1958), lactogenic hormone by the crop gland secretion of the pigeon (Riddle *et al.*, 1933) and oestrogen by the oviduct weights in chickens that have received an oral dose (Dorfman and Dorfman, 1953). A

useful summary of many methods is given by Loraine (1958) in his excellent book.

3. Other official assay methods

Digitalis and Vitamin D are the only other substances for which biological estimations are quoted.

For the assay of digitalis, guinea-pigs are now used, although for many years an alternative test was made in frogs. However, in 1950 it was shown by Miles and Perry that large numbers of frogs were needed to obtain satisfactory limits of error in the assay.

Vitamin D for human consumption is estimated in litter-mate rats as recommended by Bliss (1952). Where Vitamin D is produced for use in farm animals, and only Vitamin D_3 is active, it must be assayed in chickens.

IV. Trimming the Tally between Animals and Experimenter

Man as the experimenter is prepared to use animals in order to accumulate knowledge. This may be about the animals themselves, their physiology in its broadest sense, their diseases and their treatment, or their behaviour. It may be about the way they react to invasion, whether of chemical compounds or of parasites; this will include investigations of the metabolic paths of these foreign bodies, and also their quantitative assay. It may be knowledge of the way they react to surgical procedures that is sought, and this will entail anatomy. Much of the knowledge is sought in the hope that it may be applicable to man.

The general distribution of the species of laboratory animals used in hospital, pharmaceutical and research laboratories, and the proportion that each species forms of the total number of laboratory animals, are discussed by Lane-Petter (1961), but this does not absolve those who work with them from the responsibility of constantly reviewing both the species and strain of animal to be chosen for each task in conjunction with the information expected from work done. In many spheres facilities are already present for such constructive investigation.

Before production of each issue of the *British Pharmacopoeia* the species of animals used for official biological assay are reviewed by experts, and increasing knowledge of strain characteristics should make possible the more precise specification of the animal for each particular test.

In large-scale screening of the activity and toxicity of new compounds, long experience demands mice as experimental animals, wherever possible, with rats as close rivals. It is in the final screening tests that differences in strain responses could be applied with profit.

As for the research worker, he must choose the best animal for his job, or at least the most suitable he can afford. Only the knowledge of the differential characteristics of species and strains relating to his work will help him in his

deliberations. If the suggestions made in this chapter stimulate his search for the ideal animal they will have achieved their end.

In their book *The Principles of Human Experimental Technique* (1959), Russell and Burch have stressed the experimenter's need of discarding convention and of searching their motives in choosing their tools. The arguments in favour of such a self-examination are irrefutable.

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Chapter 10

Guinea-pigs

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I. Introduction

A. General description

Cavia porcellus, the guinea-pig, is the name given to the domesticated species of the genus *Cavia*. It belongs to the family *Caviidae*, of the order *Rodentia*, sub-order *Hystricomorpha*. It is thus more closely related to the capybara, the chinchilla, the coypu and the porcupine than to the murine or sciurine rodents.

The guinea-pig has been domesticated in South America for a very long time. Little is known of its origin; it was found, as a domestic animal, in the houses of the Andean Indians, when the first Spanish invaders arrived. It was brought to Europe in the sixteenth century and, during the next four centuries, it was kept in most European countries and in North America as a pet and for show and fancy. It has, however, like the rabbit, been used as an article of food in Mediterranean countries and in North America, and a U.S. Department of Agriculture leaflet of 1921, in addition to giving excellent advice about the breeding and management, includes a recipe for cooking guinea-pigs. During the second world war, the rural population of Italy was strongly encouraged to keep guinea-pigs, as well as rabbits, to supplement their meagre meat ration.

Scientists customarily use the name guinea-pig, in spite of its cumbrousness and the fact that the origin of "guinea" is lost in obscurity. Fanciers, on the other hand, more often use the name cavy, which would seem to have everything except tradition to recommend it. The origin of the German name, meerschwein or sea-pig, is equally incomprehensible, but it adds a third type name that has found its way into other languages.

That such a familiar animal should be so variously named; that the name used in English-speaking laboratories should be the least practical; and that there is no agreement about whether or not a hyphen should be used between guinea and pig; are mysteries that must for the time being remain unsolved.

The guinea-pig exhibits three coat types. The normal, often known as the smooth-coated English, has short, straight, rather coarse hair, which normally lies back along the body and down the limbs, and in health carries a high shine. The Abyssinian is another short-coated type, but with coarse hairs arranged in irregular rosettes over the whole body, which gives it an untidy but by no means unattractive appearance. There may be all degrees of rosette formation in English × Abyssinian crosses. Lastly there is the Peruvian or silky type, with long fine hair, several inches in length, which may show some rosetting. This impractical pelage makes the owner look like a bride who has lost her train bearers.

A new mutant, *sticky*, that affects the hair of guinea-pigs was described in 1959 by Herbertson *et al.* They found the condition to be not detrimental to survival, and to be controlled by a single recessive gene. *Sticky* guinea-pigs have an excessive amount of lipid in the hair, and the lipid differs also in kind from that present in normal guinea-pigs.

Like man and the apes, the guinea-pig lacks a tail. It is highly vocal, gregarious and surprisingly sensitive and responsive to the standard of care offered to it. It does not have the rat's enterprising and exploratory attitude towards unfamiliar food substances, and indeed may have to be persuaded to learn to eat a new variety of green food. Once the lesson is learned, however, the appetite is voracious and, unlike the rat, undiscriminating.

The digestive system of the guinea-pig is relatively inefficient. It relies on the breakdown of cellulose by bacterial action in the gut to release digestible nutrients. The character of the gut flora is therefore of crucial importance and anything that alters it profoundly may have a catastrophic effect. For example, Roine *et al.* (1953) reported that guinea-pigs fed with aureomycin (chlortetracycline hydrochloride) died. *Listeria*, a harmless gut commensal normally present in small numbers, is insusceptible to aureomycin and, when other commensals were killed by the antibiotic, overgrew to such an extent that it became pathogenic and killed the animals.

In spite of its long-standing popularity as a laboratory animal, the anatomy of the guinea-pig has been very imperfectly studied. For some reason, the much less typical rabbit is used traditionally as the type mammal for teaching zoology. It may be—one hopes it will be—that the recent interest in the guinea-pig as an excellent tool for immunogenetic research will stimulate further study of the anatomy and physiology of this most interesting animal.

B. USES

The name guinea-pig has become almost synonymous with experimental animal, although in fact mice and rats are used in far greater numbers. As early as 1780 Lavoiser used the guinea-pig for the measurement of heat production.

The guinea-pig has proved to be a most useful laboratory animal; it is prolific and easy to keep in captivity, like other laboratory rodents, but it differs from them in certain respects that are sufficiently remarkable to set it apart from them. For example, it needs Vitamin C in the diet, and it is very susceptible to tuberculosis and to anaphylactic shock.

1. Vitamin C

In 1897 Theobald Smith recorded the death of one of his experimental guinea-pigs from what appeared to be a deficiency disease resulting from a lack of green food. Only in 1907 did Holst and Frölich point to the connection between this condition and that of scurvy, which had been the plague of sailors. Since then, guinea-pigs have been used for work on Vitamin C. Guinea-pigs resemble primates, including man, the flying fox (an Indian fruit-eating bat) and, among birds, the red-vented bulbul in requiring Vitamin C (ascorbic acid) in their diet; without it, like man, they will die. Indeed, a guinea-pig needs almost as must Vitamin C as a man for the maintenance of good health.

Until chemical methods of assaying ascorbic acid were developed, guinea-pigs were the most convenient animal with which to assay this vitamin.

2. Tuberculosis

Guinea-pigs are very susceptible to tuberculosis, of both human and bovine types. The course of the infection in the guinea-pig resembles a primary

progressive infection in man. Massive airborne infection through the lungs will lead to a rapidly spreading miliary pulmonary tuberculosis, killing the guinea-pig in less than a month. The infection most commonly seen, however, is the result of inoculation of tubercle bacilli in samples of milk, sputum or other material; or, occasionally, an accidental but very light airborne infection. In both these cases there is likely to be a bacteraemia, with the excretion of live tubercle bacilli in small numbers in the urine and faeces after 5-10 days. Infection of lymph glands draining the area of primary inoculation provokes an inflammatory reaction, with enlargement and caseation of the glands, and eventual spread throughout the body. Recognizable and diagnostic lymph gland reaction is unlikely to be seen in less than 4-6 weeks, when the animals may be killed and examined post mortem. The sensitivity of the guinea-pig to tuberculosis is such that only a very few bacilli are needed to infect, and fatal generalized tuberculosis is the inevitable outcome.

Guinea-pigs, therefore, have played, and still play, an essential part in the diagnosis of tuberculosis in man and cattle. There is evidence that some strains of guinea-pig are more susceptible than others to tuberculosis, in that the characteristic lesions develop in them more rapidly after inoculation; but there is no evidence of the existence of any strain that is resistant, in the sense of not eventually developing the disease after inoculation with live bacilli.

3. Anaphylaxis

Guinea-pigs are peculiarly susceptible to anaphylaxis, and also to other allergic manifestations. Smith *et al.* in 1961 reported an interesting example of the sensitization of guinea-pigs to milk. The guinea-pigs, coming from an accredited commercial breeder, had been fed on a diet containing a dried milk product. When they were subsequently inoculated with samples of milk (as a test for the presence of tubercle bacilli) they died in anaphylactic shock. We have had similar reports from other sources, confirming that dried milk in the diet is likely to sensitize guinea-pigs in this way. On the other hand, liquid milk fed to them seems to have no such effect, and this has recently been confirmed by Boycott (1962, personal communication). Moreover, many American workers studying the nutrition of the guinea-pig have included whole milk in their diets, but have not reported sensitization. It is not known, however, whether the guinea-pigs used by them were challenged by inoculation with tuberculous material.

The work of Moore (1957) on susceptibility to salmonella infection may be relevant in this connection. He found that fewer organisms were needed to infect a guinea-pig by the conjunctival route than by ingestion, by a factor of not less than a million. This suggests that the mucous membrane of the eyes, and possibly of the nose, may be more easily penetrable by whole proteins or protein aggregates than might be expected. The dust from diets containing

dried milk would readily "infect" guinea-pigs, in the sense of gaining entry into the body, with the milk proteins unaltered, and so sensitize the animals; proteins from liquid milk, on the other hand, would be unlikely to enter in the same way.

4. Laboratory investigations

In recent years in Great Britain about 60% of all guinea-pigs used in laboratories have been used for diagnosis of disease in man and animals and, of these 60%, two-thirds have been used in connection with tuberculosis. The remaining third is shared between work on brucellosis, diphtheria, anthrax and syphilis (indirectly by the provision of complement for the Wasserman test), in that order, and a miscellaneous list of other tests.

Twenty per cent of guinea-pigs have been used for bio-assay purposes, including the testing of drugs, in chemotherapy, pharmacology and toxicology, vaccination procedures, anaphylaxis, toxicity tests and other purposes, in that order.

The remaining 20% have been used in research, mainly bacteriology, pathology and parasitology.

Guinea-pigs will continue to find an indispensable place in the laboratory, because of the many special characteristics that they possess. The greatest demand is in the diagnosis of tuberculosis and, therefore, as has been pointed out elsewhere (Lane-Petter, 1961, pp. 9-10), the prosecution of a vigorous campaign against tuberculosis will be accompanied by a large increase in this demand.

II. The Environment

Guinea-pigs are reasonably hardy animals and do not demand a lot of coddling. In a temperate climate they can be bred in unheated quarters, even semi-outdoor hutches, all the year round, but in these conditions it will be found that the breeding is depressed in the late autumn and winter, producing the minimum number of young at just the time of year when the laboratory demand is at its maximum. This effect may be mitigated to some extent by providing enormous quantities of bedding in the form of hay or straw, inside which the local temperature is raised considerably, but this is not an acceptable solution under laboratory conditions. Artificial heat, in the temperate zone, is the only practical solution in winter time. In tropical or sub-tropical conditions the necessity for full air-conditioning, or some other efficient method of cooling, is inescapable during the hot season.

A. Temperature, ventilation and humidity

The guinea-pig has a light pelage and not much subcutaneous fat. It has, however, a compact body, which serves it well for heat conservation but badly for heat dissipation. It is, in fact, more likely to suffer from heat than cold.

A temperature range of 18-21°C (64·4-69·8°F) should be aimed at for guinea-pigs. Temperatures as low as 14-15°C (57·2-59°F) will do no great harm to a healthy colony, even in the absence of copious bedding, provided there are no draughts or rapid fluctuations of temperature. On the other hand, a sustained temperature of 25°C (77°F) with a high humidity and little or no air movement is likely to cause guinea-pigs great distress and may lead to death from heat exhaustion, premature parturition, death of young, and failure of lactation. Overcrowding and the use of the more enclosed type of cage will enhance susceptibility to the effects of heat.

Guinea-pigs, like most laboratory animals, do better when they have ample fresh air. Their wild ancestors were probably surface-dwelling animals, with only shallow burrows and concealing themselves in the tangled undergrowth of grassland. The requirements for ventilation of guinea-pigs' houses are therefore quite simple. There should be a sufficient number of changes of air to keep the atmosphere fresh at all times; that is from two to six complete changes per hour, depending on the density of the population in the rooms. Provided the ventilation of the room extends to all parts of it, leaving no stagnant pockets of air, and provided the cages are not so enclosed as to prevent the free circulation of air between them and the ambient atmosphere, this is enough.

Draughts are to be avoided, as much for guinea-pigs as for any other laboratory animal. A sudden drop in temperature, which is likely to follow the injudicious opening of a window in the teeth of a cold east wind, will be likely to lead to several cases of pneumonia, the causative organisms of which (usually pneumococcus or streptococcus) are frequently present in the immediate environment.

On the other hand, in hot weather increased ventilation may be necessary, if only to provide the air movement to enable the guinea-pig to lose heat. Heat loss is chiefly by convection, although evaporation contributes a little to it. Stagnant air with a high humidity is inimical to heat loss. Heavily pregnant sows are especially susceptible to heat exhaustion, and in uncomfortably hot conditions may be seen prostrate, breathing rapidly and evidently distressed.

B. CAGING

Guinea-pigs for laboratory use will always be kept in some sort of building that is reasonably proof against rats, mice and crawling insect pests. It should, however, be proof also against flying insects and against birds, some of which can cause dangerous infections (for example, pasteurellosis).

1. Hutches

Private breeders of guinea-pigs have traditionally used hutches for breeding and for holding guinea-pigs. Since such breeders have for many years supplied

most of the guinea-pigs for laboratory use in Great Britain, and still account for nearly half the total demand, it is not surprising that until recently hutch type accommodation was often found in laboratories. Such caging was sometimes of wood, with or without a metal or other impervious lining to the floor and lower part of the walls, and this raised insoluble problems of hygiene, especially if the cages were permanently built into the structure of the room. Elsewhere, there have been permanent structures of concrete, in several tiers, with metal or wooden frame doors.

Today such equipment must be regarded as quite obsolete. It is virtually impossible to disinfect it satisfactorily, for all the antiseptic that may be slopped around, and it has few if any contributions to make to the saving of labour. A virtue originally claimed for the built-in hutch, whether of wood or of concrete, was its durability. Those who now have the task of dismantling such erections may be pardoned for doubting that this is a virtue.

The only place for hutches is with the small breeder raising guinea-pigs in semi-outdoor conditions, where some protection from the weather is essential and ventilation is no problem. Backyard breeders of this kind still have a useful part to play in supplying small laboratories with guinea-pigs. Some British breeders, apparently against all the rules of hygiene and the expectations of scientists, are capable of raising consistently healthy animals all the year round, but their methods are not usually to be recommended for emulation.

2. Floor colonies

If the ancestral guinea-pig was mainly a surface dweller, it is to be expected that the laboratory descendant should adapt easily to life on the floor of the animal house. The floor pen, the historical successor to the hutch, has been described by many writers (Hughes, 1947; Weitz, 1954; Paterson, 1957) and widely used.

Guinea-pigs kept in floor pens are placed directly on the floor, which has a covering of bedding material—sawdust, peat moss, straw, hay. The pens are separated from each other by low partition walls, usually movable. Some groups of animals comprise as many as twenty-five adults, together with their progeny up to weaning age.

Various opinions have been expressed about the permissible density of guinea-pigs in floor pens (as well as in cages). The serious observations that have been made on this subject do not, however, show very close agreement. Some authorities have recommended a density of up to ten adult guinea-pigs per square metre (about one per square foot); others have been equally emphatic that a density of six per square metre (one per 1·7 ft^2) is the maximum. The higher density is probably acceptable—that is, ten adults per square metre—provided that extra allowance is made for the young, at the rate of about one square metre for thirty young.

Floor colonies are certainly an advance on hutches, because they lead to great saving in labour. The animals are more easily observed, and the conditions might be regarded as more closely simulating their natural environment—if that, indeed, is a valid argument. But there are many disadvantages in this system. To begin with, floors are almost as hard to disinfect as built-in hutches, and everything settles on the floor. Then the lowest foot or so of any room is the hardest to ventilate evenly—more so if it is broken up into pens by walls a foot or more high—and it is the air next to the floor that the animals are breathing. The rest of the room is not utilized, thus representing a great extravagance of space, which is especially uneconomic in a heated guinea-pig house.

Lastly, floor colonies exhibit in extreme degree an inevitable disadvantage of any solid-floored living area for guinea-pigs. Every time the animals run about they stir up dust, from bedding and excrement alike, and this dust is inhaled freely and also settles in the nose and on the conjunctivae, both ready routes of infection (Moore, 1957). In a group of some twenty guinea-pigs in an area of, say, two and a half square metres there is plenty of space for running about, even for stampeding, which guinea-pigs are especially liable to. It can hardly be considered desirable that the animals should be almost continually subjected to breathing bacteria-laden dust. In fact, a single guinea-pig infected with salmonella would certainly spread his infection to most of the colony in a short time.

3. Batteries

It has been found that guinea-pigs will normally do very well on wire-floored cages; sows will farrow, and young animals grow up, quite satisfactorily without ever putting a foot on solid ground. The key word here is *normally*. Many breeders have raised guinea-pigs on wire-mesh floors and gone from year to year with never a broken leg. Others have been less fortunate—or perhaps less skilful.

Two things are necessary if wire floors are not to lead to broken legs. The first is that the guinea-pigs shall be brought up to living on wire, and shall not be liable to stampede. The second is the right size of wire mesh.

Guinea-pigs brought up on wire very soon learn to walk on it without trouble. If, however, they have been reared on solid floors, and are subsequently transferred to wire, it is wise to cover the wire with a generous amount of bedding. By the time this has been eaten or pushed through the wire mesh, the animals will have acquired the habit of wire walking, and no trouble will follow.

Stampeding is a hazard that will nearly always lead to injury; broken legs on wire are only one of the unfortunate results of stampeding; another being the overrunning of young. A colony of guinea-pigs when suddenly disturbed may run frantically round the cage or pen, scattering young animals and

pregnant sows on all sides, in a sort of circus or whirlpool motion. It is a panic reaction to fright, and it is infectious. Stampeding in one cage will cause animals in other cages to follow suit. A colony that, through good management, never stampedes, may acquire the habit from newly introduced stock which have the habit. This condition is both preventable and curable; sympathetic and skilful care will always eliminate it. It follows that stampeding is a sign that care and management in the animal house are not as good as they should be.

Wire-mesh floors are to be preferred for guinea-pigs, because they will for all practical purposes cut out cross-infection by intestinal organisms. The mesh, however, must be large enough to let the droppings through, but not such as to catch legs and break them. Many sizes have been tried. A mesh small enough (e.g., 8×8 mm) to prevent legs getting through may also hold the faeces. A slightly larger mesh (12×12 mm) will let faeces through, but will catch the legs of new-born young, before they have learned to walk on wire. What usually happens is that the foot goes through one hole, and the claws come up through the adjacent hole and catch on the wire (Fig. 1). The animal, in attempting to get free, twists its leg and fractures the bone.

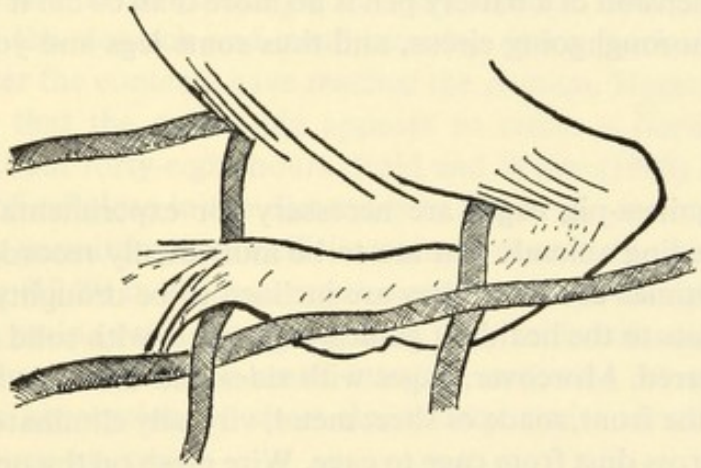

FIG. 1. If the wire-mesh floor of a guinea-pig cage is the wrong size, the animal's foot can get caught in it, which may lead to a broken leg.

The answer is a mesh so large that feet will go through and come out again, without getting caught. In practice it is found that a mesh of about 18×18 mm is satisfactory; even better is an oblong mesh of about 75×12 mm. The wires should be straight, not crinkled, and welded at their intersections; in the case of the oblong mesh, the longitudinal wires (that is, those as 12-mm intervals) should be on top.

Even with mesh of this size, it is possible to get broken legs. But if they do occur, the fault lies elsewhere; not in the method, but in the management.

If these qualifications about the use of wire floors are borne in mind, it is

possible to breed and hold guinea-pigs in many-tiered batteries, with wire-floored pens that are virtually self-cleaning. The droppings fall through the wire on to a tray, which forms the roof of the tier below. The tray may be covered with bituminized paper, drawn through every day or two from a roll at one end; or with a sheet of plastic, which is easily cleaned down either manually or mechanically.

Such a method can show a gratifying economy of labour. A battery of this type, has been dsecribed by Lane-Petter (1954) and has worked well in practice, but better devices embodying essentially the same principles have since been developed and successfully used.

One or two details require attention in the design. The wire floor must be such that it offers no lodgement for droppings anywhere—at the corners, along the edges, or at the site of intersection of reinforcing wires. If lodgement can occur, most of the advantage of wire floors disappears.

The dimensions are also important. A group of ten or twelve adult guinea-pigs is about as large a group as it should be necessary to keep together. These will occupy 1-1·2 m^2 of floor space; or, if they are a breeding colony, twice that space. The pen should not, however, be square in shape, because of the tendency of guinea-pigs to run round in circles when they are panicked. If the short dimension of a battery pen is no more than 60 cm it will be difficult to develop a thoroughgoing circus, and thus some legs and young lives may be saved.

4. Small cages

Individual guinea-pig cages are necessary for experimental purposes, as well as for breeding animals that are to be individually recorded. Wire-mesh cages are sometimes used, but they are inclined to be draughty and draughts do not contribute to the health of guinea-pigs; cages with solid sides and back are to be preferred. Moreover, cages with sides and back, and even with the lower third of the front, made of sheet metal, virtually eliminate the scattering of faeces and gross dust from cage to cage. Wire mesh on the upper two-thirds of the front, and on top, will provide ample ventilation if the room itself is properly ventilated.

Floors of individual guinea-pig cages may also be of wire mesh; or they may be solid, with the cage standing in a sheet-metal tray. With a solid floor, however, there is the danger of the animal scattering dust as it moves about the cage. If the animal has been inoculated with a specimen of milk or sputum containing live tubercle bacilli, the particles of dust, which are excellent vehicles for the bacilli, will escape from even the most enclosed type of conventional cage, to the peril of other guinea-pigs and above all the staff who work in the animal house.

It is therefore safer and also more hygienic to keep experimental guinea-pigs in cages with wire-mesh floors, through which the droppings can fall.

If there is a chance that the droppings can contain living and dangerously pathogenic organisms, then the tray below must be covered with peat moss, sawdust or other absorbent material kept permanently moist, preferably with a suitable disinfectant (see the report of the Public Health Laboratory Service, 1958).

There would at first sight seem to be no practical reason why individual cages for guinea-pigs, or small cages to house perhaps two animals, should not be built in batteries on the same principle as those used for rabbits (see Chap. 11). It could prove to be labour-saving if large numbers were kept. One serious disadvantage, however, would be the difficulty of adequately sterilizing the individual compartments. This could be a fatal drawback in an animal susceptible to several bacterial diseases and also used in the diagnosis of some of them. Perhaps, after all, the individual cage is the most useful for general experimental purposes.

III. Feeding

A. DIGESTION

Reid and White (1948) studied the rate of movement of contents through the digestive tract of the guinea-pig and showed that there is a rapid movement through the stomach and small intestine and a marked retardation of movement after the contents have reached the caecum. Hagen and Robinson (1953) report that the guinea-pig appears to retain a portion of ingested material for about forty-eight hours. Reid and White (1948) also found that the presence of cellulose in the diet tends to retard the movement of the gut contents, thus permitting more efficient absorption of vitamins, as suggested by Mannering (1949) in *Vitamin requirements of the guinea-pig*. The absorption of sugars, amino acids, fats, long-chain fatty acids, vitamins and probably minerals occurs chiefly in the small intestine and, to a lesser extent, in the stomach. The absorption of short-chain fatty acids occurs chiefly in the caecum and large intestine.

After passing into the caecum, progress of the gut contents is slowed down considerably. It is in this organ and in the large intestine that roughage or bulking material exerts its major effects. Reid (1958) found that whilst the mechanism by which these bulking agents function may not be clearly understood, it seems probable that they promote the growth of micro-organisms which synthesize essential factors. Hagen and Robinson (1953) found that fermentation occurs with the formation of short-chain volatile fatty acids which appear to be absorbed through the wall of the caecum and the large intestine.

The caecum in the guinea-pig is a large organ constituting about 15% of the total body weight. Phillips *et al.* (1959) report that the caecum of germ-free guinea-pigs is disproportionately large, and the wall is very thin and more

elastic than that of the conventional guinea-pig, whose caecum has a tough, thick wall. The actual cause of caecal distension in the germ-free animal has not been established, but it appears to be a lack of resistance to stretching on the part of the caecal wall and is present, presumably, in all species of germ-free animal. These workers are inclined to regard caecal distension as an abnormality and believe that further study on the rearing of germ-free guinea-pigs warrants consideration.

B. REQUIREMENTS

Paterson (1957) states that "Cavies are vegetarians by nature and they thrive on a wide variety of diets provided care is taken to include the necessary vitamins, particularly C and E, and hay to provide a certain unknown, but essential, factor or factors. A satisfactory diet will consist of a concentrate, in either mash or pellet form, green food in some form, hay and water." In addition to proteins, carbohydrates and fats, a number of other dietary constituents are necessary for the maintenance of health in the guinea-pig; these include roughage, vitamins, minerals and water.

The modern method of feeding guinea-pigs is to provide them with a diet in pellet form which can be presented in self-service feeding hoppers. Most pelleted diets used in Great Britain are not in themselves complete diets; they have to be supplemented with hay to provide roughage, and with either fresh green food or ascorbic acid. Fresh green food provides the necessary Vitamin C; it is not just an uneconomic vehicle for water.

1. Hay

Hay is a source of roughage; many workers have studied the amount of roughage which must be present in a guinea-pig diet to give satisfactory results.

Woodward and McCay (1932) in their study of synthetic diets for herbivores drew attention to the high intake of cellulose. Hogan and Ritchie (1934) fed guinea-pigs semi-synthetic diets containing 3-15% cellophane; 3% was unsatisfactory, 15% produced a degree of success. Crampton and Bell (1947) found that while cellulose was a valuable addition to the diet of the guinea-pig, it was not essential. Booth *et al.* (1949) investigated a wide variety of bulking materials and found that these materials improved growth. Heinicke and Elvehjem (1955) studied the effects of high levels of fat, lactose and type of bulk in guinea-pig diets; they found that 15-18% of bulking materials in the diet gave satisfactory results.

Paterson (1957) noted severe and rapid dental overgrowth in nursing females deprived of hay. Rapid dental overgrowth in guinea-pigs could, as Paterson suggests, be of dietary origin. Some pelleted diets have a preponderance of phosphorus while hay, although a variable source of some minerals, is a good source of calcium; depriving animals of hay upsets the calcium-phosphorus ratio to the detriment of health. Dental overgrowth is partly

controlled by the animals indulging in what has been erroneously described as pseudo-rumination, but is in fact grinding of the teeth, a normal and necessary activity in the rodent.

TABLE I

Guinea-pig diets

Used in Great Britain				*Used in U.S.A.*			
Diet 18 (Bruce and Parkes, 1947)		SG1 (Short and Gammage, 1959)		Rockland Guinea-pig diet		Purina Guinea-pig chow	
FORMULAE							
	%		%		%		%
Bran	15	Best white fish meal	10	Ground whole oats		Ground wheat	
Barley meal	20	Grass meal	20	Ground whole wheat		Ground yellow corn	
Ground-nut cake	15	Bran	40	Dehydrated alfalfa meal		Dried skimmed milk	
Linseed cake	10	Middlings	18	Soyabean oil		Soyabean meal	
Dried meat and bone meal	8	Sussex ground oats	12	Irradiated brewers' yeast		Cottonseed meal	
Dried grass meal	30			Steamed bone meal	1	Alfalfa meal	
Calcium	1			Calcium carbonate	1	Cane molasses	
Sodium chloride	1			Iodized salt	1	Wheat middlings	
						Animal fat (preserved with BHA)	
						Supplements	
						Vitamin B_{12}	
						Riboflavine	
						Ca pantothenate	
						Niacin	
						Ascorbic acid	
						Vitamin A oil	
						D-activated plant sterol.	
						Vitamin E	
						$CaCo_3$	2
						Low fluorine rock phosphate	1
						Iodized salt	0·5
						Manganese sulphate	0·02
						Zinc oxide	0·02
	100		100				
CHEMICAL ANALYSIS							
Crude digestible protein	16·5	Protein	14·692	Protein	17·66	Protein	24·62
Fat	4·6	Fats	3·4	Fat	2·84	Fat	4·20
Soluble carbo-hydrate	33·7	Carbohydrates	36·6	Carbohydrates	73·61	Nitrogen free Ext.	40·88
Fibre	6·7	Fibre	4·5	Nitrogen free Ext.	60·52	Fibre	10·40
		Ash	7·3	Fibre	12·90	Ash	8·50
		Lime	1·3	Ash	8·34		
		Phosphoric acid	2·5				
		Potash	1·5				
		Chlorine	0·43				

2. *Vitamins*

a. *Vitamin C.* Paterson (1957) states that "A growing guinea-pig weighing 250 g requires 3·0 mg, and a fully grown resting pig at least 10 mg, of vitamin C daily to prevent the occurrence of scurvy, to maintain natural resistance to disease and also to keep the blood complement at its normal level." Brown *et al.* (1953) carried out an experiment in which they fed one group of guinea-pigs an amount of ascorbic acid equivalent to that ingested by a control group which were fed cabbage *ad lib.* The mean intake of ascorbic

acid from the cabbage was 45 mg per day. There was no difference in intake of cabbage between males and females, except during pregnancy when the intake of the female increased slightly. These workers showed that the animals obtaining ascorbic acid from the cabbage grew more rapidly than those which received synthetic ascorbic acid, suggesting that cabbage is more than a mere vehicle for ascorbic acid. Bruce (1950) gave the guinea-pig 5 mg ascorbic acid daily during the fast growing period and 20 mg per day during the period of reproduction. She found that the substitution of ascorbic acid for fresh green food had no effects upon growth rate or reproduction. This discrepancy may be due to the fact that Brown and his co-workers appeared not to have given their animals hay. Bruce stated that diet 18, which both Brown and Bruce used as their basic diet, did not contain sufficient vitamin E, but both vitamin C and E requirements were fully met by feeding fresh green food and hay, the latter also supplying the necessary roughage.

Vitamin C may be provided by the addition of ascorbic acid to the animals' drinking water. Paterson (1960) found from experience that it was necessary to add 0·2-0·4 mg ascorbic acid per ml of water to ensure the animals received an adequate amount. The water used should be either resin-column de-ionized or distilled, and 0·2% of citric acid should be added. There are three other important points which must be observed. The mixture must be freshly prepared daily; the occasional animal will refuse to drink the laced water; and it is most inadvisable to allow the prepared water to come in contact with a metal surface—this applies particularly to the brass alloys frequently used for making drinking tubes, since the copper present in them rapidly oxidizes the ascorbic acid. Glass drinking-tubes must be of stout Pyrex glass, because guinea-pigs will chew and crush soda glass and injure themselves.

Sprouted grain may be used as a source of vitamin C although it does not have the same high content as certain other field greens. McCance and Widdowson (1960) give the figure of 60 mg per 100 g for cabbage and 100 mg per 100 g for Brussels sprouts. Cammiade and Lane-Petter (1953), describing a method of producing sprouted grain within the laboratory, reported that the vitamin C content was 28 mg per 100 g. This figure is considerably lower than the figures given for other types of green food; there is however a good case for feeding sprouted grain in preference to field greens.

It can be produced with modest equipment at a reasonable cost, as is demonstrated at the State Serum Institute, Copenhagen, where a room is set aside and maintained at a temperature of 20-28°C (68-82·4°F), the only necessary equipment being racks and perforated trays. Dr. Vlček in Czechoslovakia produces sprouted grain in a normal greenhouse where it is grown on a damp peaty soil. In Czechoslovakia and the U.S.S.R. field greens are not available during several months of the year, but in both countries good guinea-pig colonies are maintained throughout these months by feeding sprouted grain. One of us (W.L.-P.) has seen a heated glasshouse near

Leningrad, where winter temperatures go down to −25°C, producing over 300 kg of sprouted grain daily for small animal feeding.

Another advantage of sprouted grain is that there is no seasonal fluctuation, either in quality or in supply, and there is virtually no danger of introducing an infection.

b. *Vitamin A.* The animal's requirements are normally satisfied by a liberal intake of carotene, a constituent of its normal diet. The guinea-pig has a low capacity for storing this vitamin, and the maintenance of good health requires its frequent ingestion. The quantitative requirement for vitamin A, either for growth or for reproduction, has not been determined. Bentley and Morgan (1945) found that 2·0 mg daily per kg of body weight resulted in the storage of vitamin A in the livers of depleted animals.

c. *Aneurine* (B_1). Slanetz (1943) determined the thiamine values of several guinea-pig diets, all of which appeared to be adequate with respect to their thiamine content. The range of values was from 0·40 to 0·65 mg per 100 g of diet. The thiamine requirement for growth has been found to be between 0·6 and 0·8 mg per 100 g diet when a purified diet was used.

d. *Niacin.* This vitamin is essential. Harris (1939) found that when he fed his animals a cooked diet made up to a Goldberger formula, growth and survival rate improved when niacin was added.

Reid (1958) gives 10-20 mg per kg diet as a minimal requirement; the niacin content of the guinea-pig's normal diet appears to be adequate.

e. *Choline.* Cassleman and Williams (1954) reported that the addition of choline to a diet presumably already containing some choline stimulated the growth of guinea-pigs, yet the dietary choline requirement of half- to full-grown guinea-pigs has not definitely been shown, nor has the qualitative requirement for reproduction. Reid (1955) found that 0·10 to 0·15 gm per 100 g diet supported 100% survival and maximum growth.

f. *Pantothenic acid.* Maynard (1951) found that the requirement of this vitamin appears to be between 1·5 and 2·0 mg per 100 g diet for very young animals. Reid and Briggs (1954) give a similar figure. The normal diet appears to contain an adequate amount of pantothenic acid.

g. *Folic acid.* The natural diet of the guinea-pig consists of mixed succulent herbs high in folic acid. It is therefore not surprising that the young guinea-pig appears to have a higher folic acid requirement than other laboratory mammals.

Mannering (1949) stated that a supply of 100 μg per day was essential for maximum growth. The requirement for reproduction has not been determined. May *et al.* (1953) suggest that the folic acid requirement for the guinea-pig is increased when there is a deficiency of ascorbic acid.

h. *Pyridoxine* (B_6). The quantitative requirement of pyridoxine for the guinea-pig has not been determined. Reid (1958) suggests something in the region of 1·6 mg per 100 g diet, and this is present in the normal guinea-pig diet.

i. *Biotin.* There is no evidence that biotin is required by the guinea-pig (Reid, 1958). Its needs are probably satisfied by intestinal synthesis.

j. *Inositol.* If the diet is a well-balanced one containing the necessary amino acids, a dietary source of inositol may be unnecessary. Reid (1958) reported that in the absence of dietary inositol the animals shed their hair and there was a dermatitis at the margins of the ears. This is a common syndrome in pregnant animals; it does not appear to be detrimental to health, but it is possibly due to inositol deficiency.

k. *Vitamin B_{12}.* Wolff *et al.* (1951) studied the distribution of vitamin B_{12} in the organs of guinea-pigs. Reid (1958) found that under well-balanced conditions of nutrition the requirement of Vitamin B_{12} is probably adequately supplied by bacterial synthesis in the gastro-intestinal tract. Intestinal synthesis may not be sufficient to ensure maximum growth under certain conditions; the diet should therefore contain 4-6·5 mg per kg diet.

l. *Para-amino-benzoic acid (PABA).* Woodruff *et al.* (1953) found that PABA is connected with the dietary supply of folic acid. It is not required when folic acid is adequate, but if the supply of folic acid is inadequate PABA exerts a beneficial effect.

m. *Vitamin D.* Coward (1952) says that guinea-pigs require vitamin D only if the Ca : P ratio is very different from 1 : 1. However, the quantitative requirement for vitamin D in diets with unbalanced proportions of calcium and phosphorus has not been determined. Many workers have tried to produce rickets in the guinea-pig, but without throwing much light on whether or not this vitamin is necessary.

n. *Vitamin E.* Several workers have studied the effects of vitamin E on muscular dystrophy. Shimotori *et al.* (1939) clearly demonstrated that dystrophy in guinea-pigs could be ascribed to lack of α-tocopherol. Their study of the relationship between vitamin E and reproduction had to be abandoned because the animals failed to survive long enough on the diets for the experiments to be carried out. Pappenheimer and Schogoleff (1944) found it difficult to study the effects of a vitamin E deficiency on reproductive functions because the animals usually succumbed before reaching sexual maturity. Ingelman-Sundberg (1949), however, found that a daily intake of 1·6 mg of α-tocopherol is necessary to support normal pregnancy. The normal guinea-pig diet appears to contain adequate amounts of vitamin E.

o. *Vitamin K.* There is little evidence that vitamin K is essential for the growing guinea-pig. It is necessary for normal reproduction, as shown by Hamilton (1943) who found that failure to supply the vitamin during pregnancy resulted in stillbirths or death of the young soon after birth. Reid and Briggs (1954) found that a supply of 0·2 mg per 100 g food appeared adequate for growth and reproduction in the first generation, but in the second generation resulted in stillbirths or death of the young soon after birth, obviously from vitamin K deficiency. Hamilton recommended 50 mg per kg diet,

which he found to be adequate, and this appears to be present in the normal guinea-pig diet.

3. Minerals

Guinea-pigs in common with other herbivores are accustomed to a high intake of minerals. Commercially produced pelleted diets are said to contain 7-9% minerals, but there is little evidence that all diets do in fact contain these percentages and in the correct proportions. Calcium, phosphorus, potassium, sodium, magnesium and chlorine are all required by the guinea-pig. Reid (1958) stated that the actual quantitative requirements have not been determined; she also adds manganese, copper, zinc and iodine to the list of essential minerals. Cobalt, being a constituent of vitamin B_{12}, is undoubtedly required for the intestinal synthesis of this vitamin.

Roine *et al.* (1949) have demonstrated the importance of potassium and magnesium in the nutrition of the guinea-pig. They found that an increase in growth was due to potassium and magnesium contained in alfalfa ash which was fed to the guinea-pig.

Reid (1958) has shown that guinea-pigs thrive on a wide range of dietary calcium, but found the Ca : P ratio to be of more importance than the actual amount of each element in the diet. A balanced relation between calcium, magnesium, potassium and sodium is also important. Hogan and Ritchie (1934) found that when the diet contained an excess amount of phosphate the growth rate was slow, with stiffness of the joints, a high incidence of calcium phosphate deposits and a high mortality rate. These conditions were most severe when the diet contained 0·09% calcium and 1·70% phosphorus. In their studies on the effects of milk diets on guinea-pigs Wulzen and Bahrs (1941) report a similar condition with deposits of tricalcium phosphate under the skin, in the joint regions and indiscriminately on many body organs. In this case they fed their animals fortified skim milk powder plus wheat straw. Paterson (1957) found many animals, especially males, suffering from soft tissue calcification, the histological picture of which suggested a similarity to hypervitaminosis D. Reid (1958) reports a personal communication from Hogan in which he concludes that the deposits are the consequence of an improper balance in the intake of calcium, potassium, phosphorus and, especially, magnesium.

4. Water

It has long been a superstition amongst backyard breeders that rabbits and guinea-pigs will develop distended abdomens if they are given water to drink. Kennaway (1943) drew attention to this superstition and pointed out that rabbits and guinea-pigs have no special capacity for surviving without water. Bruce (1950) has shown that an 800-g guinea-pig being fed 30 g green food per day also drinks an average of 84 g of water per day; she concluded,

therefore, that the average daily requirement of drinking water was 105 g per kg body weight. Brown *et al.* (1953) studied the rearing of guinea-pigs on a diet containing synthetic ascorbic acid; they found that the control group, fed fresh cabbage, consumed 40-70 g of cabbage daily and drank 68 ml water daily. Ellinger (1956) made a study of the drinking habits of guinea-pigs weighing 250-260 g. They were kept on a dry diet and, presumably for this reason, their average daily intake of drinking water was 83·6 ml. It would appear that for practical purposes 10 ml of water is required for every 100 g body weight of guinea-pig.

5. Grass Juice Factor

Many workers have drawn attention to a so-called grass juice factor essential in guinea-pig nutrition. The belief that such a nutritional factor exists has developed chiefly from observations carried out on synthetic or semi-synthetic diets. This factor has never been identified. Hogan and Hamilton (1942) reared guinea-pigs on simplified rations and, in view of their success, were of the impression that the so-called grass juice factor was non-existent.

C. PELLET FEEDING

Unlike the rat and the mouse, the guinea-pig lacks the sort of mouth that can gnaw cubes through the bars of specially designed feeders. Compound diets are therefore generally manufactured in pellet form and fed in hoppers. Guinea-pigs have a habit of picking up their food and dropping it again, and cubes are therefore wasteful because those dropped by the animals become soiled and unpalatable. A pelleted diet is less wasteful.

One of the most avidly used British pelleted diets has recently been shown by Paterson *et al.* (1962) to have contained, on occasion, ground-nut meal which was toxic. The toxic substance has been traced to the action of a fungus, *Aspergillus flavus*, on the meal. The characteristic signs in the guinea-pig are oedema, especially in the abdominal region, and on post-mortem examination more or less severe damage to the liver, with scattered haemorrhages in many organs and tissues. The condition was first mentioned incidentally by Bruce (1950) and fully described by Paget (1954). Recovery sometimes takes place, with some degree of permanent impairment of health.

A pelleted diet is not in itself a complete diet and should only be regarded as a convenient mixture of the necessary concentrates. To complete the diet the following supplements are usually necessary: (1) hay to provide the required roughage, and possibly some essential nutrients, and (2) fresh green food, primarily as a source of vitamin C, but also perhaps to supply other essential nutrients. The complete elimination of both these supplements from a vigorously breeding colony has not yet been permanently achieved but there is reason to believe that it can be done.

The present tendency is to attempt to feed guinea-pigs on a complete

compound diet, water and nothing else. The complete diet has to contain adequate amounts of fibre and all necessary nutrients. It must also contain stabilized vitamin C, unless this is to be given in the water, which probably raises more problems than it solves. There are practical difficulties in devising such a diet, but they are being overcome. It is, however, important to consider the possibility that such a diet might suit one strain of guinea-pig but fail to support another. We know that there are strain variations of this kind in mice, and it would be surprising if they were not also present in guinea-pigs. Claims to have discovered a complete compound diet for guinea-pigs should therefore be received with acclamation, heavily moderated by caution and even scepticism.

D. PRESENTATION OF FOOD AND WATER

All guinea-pigs are likely to be offered some part at least of their diet in the form of compressed pellets, together with water, of which they need a liberal supply. The pellets are best fed in hoppers, to prevent waste, such as are described by Lane-Petter (1957) or Paterson (1957). If hay is given, it may be placed in a hay rack, but most guinea-pigs will nevertheless succeed in pulling it all out and scattering it about the cage. If food is presented in bowls, some will certainly be eaten, but the remainder will be fouled. A bowl is an open temptation to all guinea-pigs, especially young ones, to sit and defaecate in.

Water, for the same reason, should not be given in bowls; water bottles or automatic drinkers are much to be preferred. Two things must be borne in mind when offering water in this way to guinea-pigs. The first is that the guinea-pig does not lick the end of the drinking tube; rather it chews the tube with its powerful teeth, so that soda glass and soft metal drinkers are rapidly broken or worn away. Hard brass, preferably plated with chromium or nickel, or stainless steel tubes are needed. The second thing to remember is that the guinea-pig is an untidy drinker. It not only dribbles as it drinks from a tube, but it also blows back up the tube, thus fouling the water in the container. Bottles therefore need frequent cleaning, and automatic drinkers have to be so designed that particles of food will not put them out of action or make them leak.

E. PALATABILITY

Contrary to common belief, the guinea-pig is quite fastidious over food. Phillips and his co-workers found that the germ-free guinea-pigs often refused to eat when their diet was changed from a liquid to a solid one. We have seen guinea-pigs suffering from scurvy although they were being offered liberal supplies of green food which should have provided an adequate supply of vitamin C. The animals found the type of cabbage offered unfamiliar or unpalatable and refused to eat it, thus developing scurvy.

There has been very little work done on the palatability of diets generally offered to guinea-pigs, but it should be borne in mind that the fresher the ingredient the more palatable will the animal find it.

F. KEEPING QUALITIES

Plouvier (1953) reports on the keeping qualities of Provender Sorolabo diets. Analysis made immediately after manufacture and after 2, 4 and 6 months' storage under defined conditions showed that diets should be used within 3 months of manufacture. This undoubtedly applies to other diets which have been fortified with either a specific vitamin or some other nutrient. Bruce and Parkes (1947) point out that vitamin C added to the diet as crystalline ascorbic acid or as present in dried lucerne meal is mostly destroyed by the pelleting process and is further reduced during storage. Further investigations into the keeping properties of compounded diets are necessary.

G. CONTAMINATED GREEN FOOD

Paterson (1962) reported that he isolated *Pasteurella pseudotuberculosis* from cabbage during the period when pigeons were short of green food and were feeding on cabbages in the fields. These cabbages were thoroughly washed and then fed to the guinea-pigs, but the animals nevertheless became infected with pasteurella. When the feeding of these cabbages was discontinued and vitamin C provided in the form of ascorbic acid in the drinking water, pasteurellosis was brought under control. There is also a real danger of green food becoming contaminated by salmonella, either in the fields, whilst in store, or during transit. Green food should be well washed and then allowed to drain before being given to the animals. It is of equal importance to ensure that green food is fresh; wilted, yellowing and mouldy green food is not generally eaten by guinea-pigs, but it should never be offered.

IV. Genetics and Breeding

A. GENETICS

One of the first papers ever to be published on the genetics of the guinea-pig was that by Castle in 1906 in which he described the results of breeding for polydactyly over a period of some five years. From then on there is a gap until 1922, when Sewall Wright began a series of reports on inbreeding and crossbreeding in guinea-pigs. Virtually, the sum of our knowledge of guinea-pig genetics is contained in Wright's admirable review of the *Genetics of Vital Characters of the Guinea-Pig* (1960).

Wright investigated the effects of inbreeding and crossbreeding in twenty-three separate families, finally selecting five families for perpetuation. With rare exceptions, mating within the families was brother × sister, the animals being mated at weaning (33 days).

The characters he studied were age at maturity, fertility, weight gain, mortality amongst the young, resistance to experimentally induced tuberculosis, sex ratio, production of montrosities and coat colour. He found during the 13 years of inbreeding that there was a general decline in vigour. This was most marked in the frequency and size of litters, indicating a decline in fertility. Favourable or unfavourable conditions affected growth and mortality among the young and fertility in all its aspects, whether the strain was inbred or not. The susceptibility to experimentally induced tuberculosis was considerably less in a crossbred than in an inbred strain, but deaths in the inbred strain from other causes made analysis of the experiment somewhat difficult. Of the five families retained for perpetuation two strains are still in existence, after more than fifty generations of brother × sister mating. The progress of inbreeding from heterogeneous stock to this present stock, which has a high degree of genetical homogeneity, can be traced through the work of Loeb and Wright (1927). Bauer (1958, 1960) has shown that these two strains have now attained intrastrain histocompatibility, and he also draws attention to the fact that the multicoloured coats of these animals (white, brown and black) show no rigid pattern of uniformity or colour distribution between individuals. Wright has also found that the white spotting of guinea-pigs depends on a pair of recessive genes with major effects. The amount of white can vary from a mere trace to 100% and this variability he attributed to developmental accidents not genetical in origin.

In Chap. 6 the inheritance of coat colour is fairly fully treated.

Eaton has also reported on the genetics of the guinea-pig. He examined the influence of heredity and other factors on litter size, birth weight, mortality and weight gain of young (Eaton, 1932a). He also reviewed the effects of inbreeding in guinea-pigs (Eaton, 1932b).

For some years the guinea-pig has been used in research on homotransplantation (see, for example, Billingham and Medawar, 1958) and this has thrown some light on the degree of homozygosity of highly inbred strains.

Young (1954) found a significant difference in the relative susceptibility to tuberculosis in two strains of albino guinea-pigs. In the course of an experiment using fifteen families, he found that all members of one family showed a high resistance to tuberculosis and all members of a second family showed a low resistance. Young inbred these two families for something less than ten generations. The low resistant strain, *K*, was difficult to breed and maintain, whilst the high resistant strain, *H*, bred well. This was confirmed in our own laboratory when the two strains were transferred there in 1954. Foundation stocks of these strains were eventually distributed to laboratories in the United Kingdom, U.S.A., France and India. However, the strains were no longer being selected for greater or lesser susceptibility to tuberculosis, and it appeared that they soon lost this potentially valuable characteristic.

Frimodt-Moller (1956, personal communication) tested the *K* strain against a strain of indigenous origin maintained in his own laboratory in India and found that the local animals were even less resistant than the *K* strain imported from England.

B. REPRODUCTION

1. Sexual maturity

The female guinea-pig reaches sexual maturity at an early age. Wright (1922) found that many young females were mated by their sires before they were weaned at 33 days. Males are slower to reach sexual maturity, and although they demonstrate sexual activity at a very early age, fertile matings do not normally take place before they are 60 to 80 days old.

2. Oestrous cycle

The oestrous cycle varies slightly between strains and between individuals, ranging from 13 to 20 days in unmated animals. Parkes and Amoroso (1951) give the interval between ovulations in unmated animals as 14 to 16 days.

The actual duration of oestrus is approximately 50 hours (with slight variations) when the vaginal closure membrane is absent. The female will accept the male for up to 15 hours out of this period, generally between 5 p.m. and 5 a.m. Blandau *et al.* (1941) found that the actual period of heat increased slightly with each successive oestrous cycle, and gave an average of 31·27 hours. The state of oestrus in the guinea-pig can be diagnosed first by inspecting the female to ensure that the vaginal closure membrane is absent, and then by taking a vaginal smear. There are several methods of taking vaginal smears, but the method least likely to upset the animal is to pipette a drop of saline into and out of the vagina. The saline is then transferred to a slide for examination under the low power of the microscope. Bacsich and Wyburn (1939), using the vaginal smear technique, describe four stages of oestrus, as shown in the following table.

TABLE II

Stages of the Oestrous Cycle in the Guinea-Pig (after Bacsich and Wyburn, 1939)

Stage	Vaginal Smear	Remarks
I	Large irregular superficial nucleated cells	Heat may occur
II	A preponderance of cornified cells	Heat. Copulation possible
III	Cornified cells decreasing. Some small epithelial cells. Some leucocytes	Not on heat
IV	Predominantly leucocytes	Not on heat

As in other rodents, copulation would be most likely to occur in stage II. Ovulation occurs approximately 10 hours after the beginning of heat and

also within 2 to 3 hours post partum, when a high percentage of fertile matings takes place. Sperms remain motile in the female genital tract for about 30 hours, but the critical period when the sperms are capable of fertilizing the ova is restricted to about 15 hours.

Evidence of oestrus may be obtained by observation. An attempt at mounting by the female is a sign of her being on heat. When the female kicks the male it is evident that the period of heat has passed. Further evidence may be obtained by applying the finger test. A hand is placed over the female with two fingers between the back legs, one on either side of the genitals. A quick rhythmic clasping movement is then applied to the female, just in front of the iliac crest. If the female is on heat she will immediately adopt the mating position.

3. Gestation period

Length of gestation varies slightly between strains, and there is also a correlation between length of gestation and litter size. Goy *et al.* (1957) in their study of the length of gestation, frequency and time of abortion and stillbirth in two strains, found that the length of pregnancy decreased with the size of litter in both. Inbred strain No. 13 had a maximum of 71 days' and a minimum of 67 days' gestation, while the outbred strain had a maximum of 67 days' and a minimum of 62 days' gestation. Eaton (1932b) found that in litters of less than 62 days' gestation no young were born alive. Abortions were relatively independent of litter size, but the number of stillbirths was directly proportional. Kenneth (1953) found that the average length of gestation was 63 days with a maximum of 66 days. Parkes and Amoroso (1951) give the average length as 62 days.

Evans (1953) reported the case of an albino guinea-pig producing two litters after being separated from the male. The time between the birth of these two litters was 42 to 46 days and the time between the birth of the second litter and separation from the boar was 70 to 74 days. It would seem from this that concurrent pregnancies of different ages are possible in the guinea-pig.

C. BREEDING METHODS

1. Mating

Bischoff (1852), Hensen (1876) and Rein (1883) utilized the post-partum ovulation in mating their guinea-pigs to obtain timed embryological material. The most practical method of mating is to allow the sexes of the correct age to mix freely and mate at will, thus taking advantage of the post-partum oestrus.

Female guinea-pigs should be mated at $3\frac{1}{2}$ to $4\frac{1}{2}$ months of age. Males should be approximately 6 months. Female guinea-pigs grow considerably during their first pregnancy, which does not appear to retard them. If they

are not mated until after their rapid growth has ceased, they deposit fat which ultimately leads to difficult parturition and a high percentage of stillbirths and also reduces their fertility.

Methods of mating will depend on circumstances. If an inbred colony is being maintained, monogamous pair mating is the most convenient method. Alternatively, a number of sisters and one brother may be mated and kept together, but this may present problems of recording when the litters are born, especially if the young are required for the next generation.

Polygamous groups are usually formed for large-scale production, ten to twenty females and one male being allowed to remain together throughout their useful breeding life. The young are reared communally and weaned at a given age or weight.

Rowlands (1949) has examined the reproductive efficiency of polygamous groups, with and without separation of the females for parturition, and with a varying number of females with one or more males. He found that about 80% of all matings were effected at the post-partum heat. The number of fertile post-partum matings decreased appreciably when the proportion of females to males in the colony was greater than fifteen to one. The optimum proportion was found to be twelve females to one male. He considered that the stillbirth rate of 5% was not directly attributable to this method in which the males are left in with the females, but that the death rate in young animals reared communally in colonies on the floor was greater than in those reared in cages.

Experience has shown that a group or harem of one male and ten to twelve females is an efficient breeding group with a high proportion of successful post-partum matings and a useful breeding life for the harem of up to 18 months. With such a group it is possible to achieve an average of sixteen to twenty young per female per year, with negligible stillbirth and infant mortality rates. Larger harems demand larger pens to house them, which increases the risk of circus movements (p. 294-5) and consequent injury to the young. Other workers (for example, Paterson, 1957) prefer smaller groups, but mainly because they fit in better with the type of equipment in current use.

Average litter sizes in harems may be about four. A higher figure than this is not achieved without a sharp rise in infant mortality. When pregnant females are separated for parturition and there is no post-partum mating, it is possible to select for and obtain an average litter size of five or even six, with a very high rate of survival of young. However, the penalty is paid of getting only three to three and a half litters per female per year, as compared with about five if post-partum mating is the rule. The total number of young per female per year, therefore, is certainly no greater when the females are singled, and the labour entailed rules this method out on practical grounds.

There have been no reports of artificial insemination being carried out on the guinea-pig. Freund (1958) does, however, describe a method and an

apparatus for electro-ejaculation of the guinea-pig. He examined the semen coagulum and described the technique.

2. Weaning

Weight of young at birth is in direct proportion to the size of the litter and to the standard of nutrition. Young in litters of one and two may weigh up to 100 g each, whereas those in litters of five or six weigh as little as 45-50 g each.

Young guinea-pigs are almost self-sufficient and will consume solid food within hours of birth, but they still depend on their mother's milk for some time and, if allowed to, will continue to suckle for several weeks. This not only damages the mother's teats but also undermines her stamina.

In harems it will be seen that young guinea-pigs suckle any lactating female indiscriminately, and that the females seem to have no proprietary interest in their own young. Indeed, at the time of parturition other, non-parturient, females will take an active interest in the proceedings, helping to dispose of the placenta and membranes and cleaning up the young as they are born. Because of this indiscriminate nursing of any young that apply for milk, it is necessary to wean as early as possible, so that older guinea-pigs may not strip all the milk off the lactating females, leaving none for the newborn.

It has been our practice to wean young guinea-pigs by weight, rather than by age, thus giving the smaller animals from large litters a better chance. As soon as they reach 160-170 g they are weaned, sexed, and placed in runs for future use.

3. Discarding breeders

Breeding stock of both sexes should be discarded at 18 to 24 months of age, by which time females in both monogamous pairs and polygamous groups will have produced five or more litters and will have reached the end of their economic breeding life.

The males should normally be removed from harem breeding pens about 3 weeks after the females have produced their fourth litters. If a post-partum fertile mating has not occurred, the 3 weeks gives ample time for mating to take place at the next oestrus. The females are then allowed to produce and rear their fifth litters. It is, however, possible in a robust stock to prolong the life of the harem to 18 months or occasionally longer.

4. Culling of inferior stock

All inferior animals, adult and young alike, must be culled, that is, removed from the colony because they are uneconomical to keep and may be a source of infection for their mates. Inferior animals are those that are unthrifty, in poor health, producing small litters, or having more than an occasional stillbirth. Thus, a female who produces a large litter and rears only a small

number must be regarded as inferior. Unthrifty animals are those that show poor growth, from whatever cause.

V. Handling, Sexing, Identification, Euthanasia

A. HANDLING

When lifting a guinea-pig, one hand should be placed well up across the shoulders without applying pressure; the thumb should be just behind a front leg and the fingers well forward on the opposite side. Avoiding pressure on the abdomen, work the fingers gently under the animal and lift it clear of the floor, simultaneously placing the free hand, palm uppermost, under the hindquarters to take the weight. Pregnant sows are handled in the same manner, but with extra care. Young guinea-pigs are handled in a similar way, but it may not be necessary to take the weight by the second hand.

B. SEXING

The sexing of guinea-pigs offers no great problem at any age. The differences in the appearance of the external genitalia, which should be viewed in a good light, are well illustrated in Fig. 2. In the guinea-pig there is no need to extrude the penis by pressure (as is done in sexing rabbits) in order to demonstrate the sex.

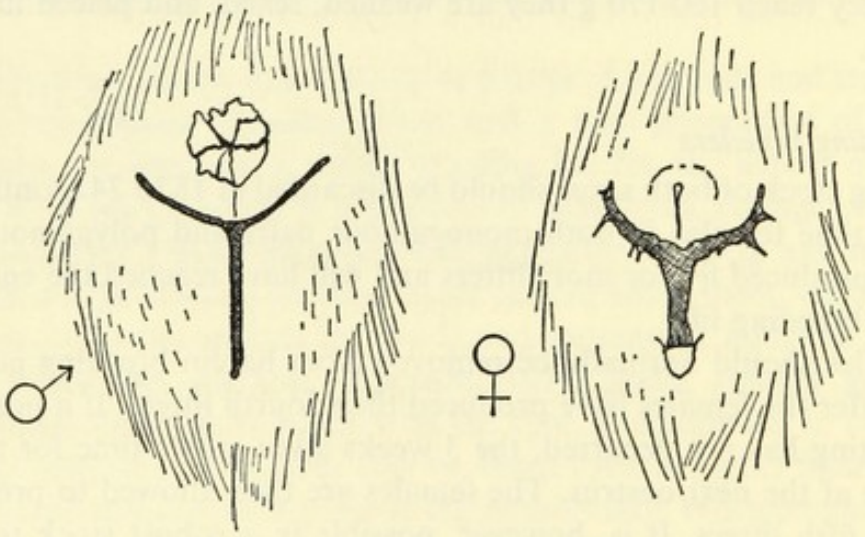

Fig. 2. External genitalia of adult male and female guinea-pigs.

C. IDENTIFICATION

1. Ear marking

Identifying guinea-pigs by punching or cutting their ears is not recommended, especially if the animals are maintained in groups; fighting may take place and the ears may be torn, thus obliterating any code of marking done in this way.

2. Ear clips

There are several types of ear clip available and all can be supplied with either numerals or letters. The tags or clips, for which a special pair of pliers is supplied, have one very sharp point which is pushed through the animal's ear as close to the head as possible. Using the pliers, pressure is then applied to lock the tag in position.

3. Staining

The following stains, which are soluble in alcohol at a temperature of 26°C (78·8°F), are generally used:

Colour	Stain
Yellow	Saturated picric acid or chrysoidine
Red	Fuchsin, either carbol, acid or basic
Violet	Methyl violet
Green	Brilliant methyl or malachite
Blue	Trypan blue

It is suggested that the stain be mixed in 70% alcohol and if possible made up in bulk, because the efficiency of the stain increases with keeping.

A small area deeply stained is preferable to a large area lightly stained, but even so certain stains, especially violet and blue, wear off quickly and re-application at regular intervals is necessary.

Before applying stain to an animal, consideration should be given to its chemical composition, the possibility of toxic effects, possible reaction on the coat and skin of the animal, and whether or not the stain may have some bearing on the ultimate outcome of the experiment.

4. Natural coat colour

Coloured guinea-pigs may be identified by their coat colour and markings. These may be marked on a stereotyped body outline (for which a rubber stamp serves excellently) on the record card, with shadings and colour indications filled in for identification. Roberts (1961) has described a method of identification by black-and-white photography which he claims is both simple and economic.

D. EUTHANASIA

Probably the quickest and most humane method of killing a guinea-pig is to stun it with a sharp blow behind the ears, and then to bleed it out. This method cannot be used when certain examinations are to be undertaken after death. We have found that the use of nitrogen is both humane and quick, and we are indebted to Dr. Jean Vinter of UFAW for drawing our attention to it. The animal is placed in a metal box with a perspex lid, fitted with inlet and outlet taps. Both taps are opened, and the nitrogen is then turned on. When the animal is seen to fall the nitrogen is turned off and both

the inlet and outlet taps closed. The use of nitrogen, being non-toxic and non-flammable, is less hazardous than the use of coal-gas.

Further advantages of nitrogen gassing is that there is no interference with subsequent post-mortem examination. Even more important, if the animal is infected with a dangerous pathogen, such as the tubercle bacillus or *Brucella abortus*, the animal is killed intact, and the danger of contaminating human workers, other animals, or the laboratory is reduced to a minimum; stunning and bleeding out or decapitation can be inexcusably dangerous procedures in such circumstances.

VI. Diseases

The guinea-pig is a hardy animal, subject to comparatively few diseases. Those of genetic origin are rare and will not be considered here. Diseases of nutritional origin, whether resulting from specific deficiencies (e.g. of vitamin C) or from the ingestion of toxic substances, occur from time to time and have been referred to in the section on feeding. Neoplasms will be mentioned but they are rare and of little practical importance. More detailed reference will be made to diseases caused by viruses, bacteria and protozoa, and to infestations with helminths and with ectoparasites. Finally, reference will be made to death from heat exhaustion. It may be mentioned here that trauma can also result in the death of young animals as a result of stampeding, or of adults, usually as a result of fighting.

A. Neoplasms

Parish (1950) states that tumours are rare, but sarcomas, adenomas and lipomas have been reported. Mammary carcinoma, malignant tumours of most organs, and leukaemia have also been described.

B. Virus diseases

The best known virus disease of guinea-pigs is guinea-pig paralysis, a progressive paralysis affecting especially the hind limbs; it is of low infectivity. It was first described by Römer in 1911 (Dumas, 1953). Other viruses that have been described in guinea-pigs are salivary gland virus, normally a symptomless infection (Cole and Kuttner, 1926); pneumopathy, also normally with few or no signs (Lépine *et al.*, 1943); and possibly the so-called guinea-pig plague described by Petri and O'Brien (1910), which is always associated with, and may be due to, infection with *Salmonella suipestifer*. Lymphocytic choriomeningitis is rarely a naturally occurring infection of guinea-pigs, but it has been reported by Blanc *et al.* (1951).

None of these virus infections seems to be of great importance in guinea-pig colonies. Indeed, the guinea-pig is somewhat remarkable in its comparative freedom from virus diseases, in strong contrast with mice, man and some other species.

C. BACTERIAL DISEASES

The main causes of intercurrent disease in guinea-pig colonies have been bacteria. Two infections stand out among all others; salmonellosis and pseudotuberculosis.

1. Salmonellosis

Guinea-pig paratyphoid may cause widespread and severe epidemics in guinea-pig colonies. The causative organisms are usually either *Salm. typhimurium* or *Salm. enteritidis*, but other salmonellas have been incriminated. The infection can remain latent in colonies indefinitely, but may be provoked into causing a major outbreak of disease by adverse environmental factors, the most important of which is probably hypovitaminosis C. In past years, when the guinea-pig's need for vitamin C in relatively large quantities throughout the year was not fully appreciated, it was customary to see outbreaks of salmonellosis regularly in the winter months, due to shortage of fresh green food. Today, such outbreaks are uncommon, but they are liable to occur during hard winters.

The signs of salmonellosis are staring coat, wasting, diarrhoea and death. The organism may be isolated from the spleen, heart blood, liver or gut, and from the faeces. It may also be found in the water bottle.

An infected colony will have to be completely destroyed if the infection is widespread and of long standing. However, a recent infection in a previously healthy colony can often be successfully controlled by systematic culling of cases and in-contacts, and a rigorous attack on poor hygiene. To be sure that such measures are always successful is difficult, but we have records of small colonies treated in this way that appeared to become clear. The subsequent history of the guinea-pigs produced in these colonies, when used for experiments in the laboratory, suggested that complete elimination of the infection had been achieved.

Salmonellosis may be completely inapparent. Some years ago a champion guinea-pig boar bred in the north of England was winning first prizes all over the country and being profitably put to stud. The animal was shown to be a carrier of *Salm. typhimurium*, but this did not deter its owner from taking it to shows and thus spreading the infection to all those with which it came into contact.

2. Pseudotuberculosis

This is normally an indolent infection of guinea-pigs, which can, however, become sub-acute, especially in the scorbutic animal. The causative organism is *Pasteurella pseudotuberculosis*. The characteristic lesions are found in the mesenteric lymph glands, which become enlarged and easily palpable in the live animal. Lesions are also found post mortem in the liver, spleen, small

intestine and occasionally elsewhere. A full description has been given by Paterson (1957). Paterson (1962) draws attention to fresh green food being a likely source of infection. It would seem from this, and from our own experience, that pseudotuberculosis is of rather low infectivity, tends to be self-limiting in a colony maintained in good conditions of hygiene, especially if the animals are run on wire-mesh floors, and is unlikely to give rise to a major epidemic unless the animals are short of vitamin C or are constantly being infected by contaminated green food.

3. Septicaemia

Acute illness and death occur in guinea-pigs from a number of virulent infections, among which the following are important: *Pasteurella septica*, rare but almost always fatal and highly infectious; haemolytic streptococci; and pneumococci. Diagnosis is made post mortem by recognition of the causative organism.

4. Respiratory diseases

Various respiratory diseases, from slight nasal discharge to pneumonia, are found in guinea-pigs. Causative organisms include *Streptococcus pneumoniae* (pneumococcus), *Bordetella bronchiseptica*, and *Klebsiella pneumoniae*. Although any of these infections may give rise to fatal epidemics, they are not common in well-managed colonies. As with virtually all bacterial diseases of guinea-pigs, a shortage of vitamin C in the diet may provoke the appearance of these diseases, and conditions of poor ventilation and heat control seem especially able to precipitate them.

5. Cervical adenitis

This is a relatively benign but not uncommon disease in guinea-pigs, caused by *Streptobacillus moniliformis*. The infection probably comes from infected hay or straw (Paterson, 1962) and causes enlargement of the cervical lymph glands, sometimes with ulceration through the skin. Eventually, in many cases, natural healing occurs, with scarring. The disease is not very contagious, but cases should be culled, and attention paid to the quality of hay and bedding being provided.

6. Other bacterial diseases

Several other bacterial diseases have been described by Dumas (1953), but they are rarely encountered in well-kept guinea-pig colonies.

D. PROTOZOAL DISEASES

1. Coccidiosis

The causative organism of coccidiosis is *Eimeria caviae*. The disease is normally uncommon, but can cause epidemic losses, especially among young stock. Good hygiene is the best prophylactic (Lapage, 1940).

2. Toxoplasmosis

Toxoplasmosis has been described by Dumas (1953); it is, however, an uncommon infection.

3. Other protozoal diseases

Dumas (1953) also described leishmaniasis, and diarrhoea due to *Trichomonas spp.* in guinea-pigs.

E. HELMINTHIASIS

Dumas (1953) describes two helminths found in guinea-pigs: *Fasciola hepatica* and *Trichinella spiralis.* Neither is common or troublesome in guinea-pig colonies.

F. ECTOPARASITES

1. Lice

Guinea-pigs are frequently found to be infested with *Gyropus ovalis, Trimenopon jenningsi* and *Gliricola porcelli,* the latter being the most common. In healthy stocks their presence is often not suspected, and it may be necessary to search carefully in the fur, especially behind the ears, for evidence of infestation. Both the lice and the nits (eggs) which are attached to the shafts of the hairs near the base, are pearly white in colour; they are difficult to detect in white-coated animals, but show up well against black or dark fur. Guinea-pigs in poor health may become heavily infested with lice which may be found all over the body, especially round the nipple area on the abdomen. There is no excuse for the presence of lice in a well managed colony. Infestation is easily treated by powdering with any of the usual insecticides, or by using aerosol insecticides in the room which Paterson (1957) recommends as being more effective.

2. Other ectoparasites

Fleas and mites can occur in guinea-pigs, but are uncommon. Treatment with insecticides and acaricides, in powder form, is effective.

G. MISCELLANEOUS CONDITIONS

Paterson (1957, 1962) mentions some further conditions that may cause losses among guinea-pigs. Among them are soft tissue calcification and pregnancy toxaemia, both of which he suggests are nutritional in origin; and dystocia, which he considers may result partly from familial predisposition and should therefore be bred out, and partly from not mating females at an early enough age, which can be corrected by better management.

We have found guinea-pigs liable to suffer profoundly and to die from

heat exhaustion in very hot weather. Heavily pregnant females are especially susceptible. In hot weather, or in hot climates, the cages should be in cool, well-ventilated areas or rooms, with plenty of air movement short of draughts, and particular care should be taken to ensure a constant supply of clean water. The water intake in these conditions is likely to be increased.

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Chapter 11

Rabbits

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I. HOUSING

The type and quality of building required to house a rabbit colony depends to a considerable extent on the objects of keeping the colony. For example, the maintenance of a breeding population of rabbits necessitates a much higher standard of accommodation than is required for a non-breeding population. It is certainly true that rabbits can be bred quite successfully in an open-sided lean-to shed, but such a system can hardly be recommended if experiments are to be carried out with breeding stock, as the breeding doe

and her litter are too exposed to vagaries of the environment. If, on the other hand, a small number of breeding stock is kept to supply stock for research purposes, there is much to be said in favour of adapting some building. Consideration must be given at the same time to the rabbit cage. It is quite a simple matter to find accommodation for rabbits which are to be housed in timber cages, but it is quite a different matter to find a suitable building if breeding stock is to be kept in wire cages. Rabbits, like most other forms of livestock, will not thrive if they are exposed to draughts. This applies equally to all ages of stock and is probably the first factor to consider when selecting a building.

A breeding colony which comprises all ages of stock should be housed according to the class and usage of the stock. This is particularly important when planning accommodation for a rabbit colony which will supply experimental material for a number of research workers. Figure 1 shows the

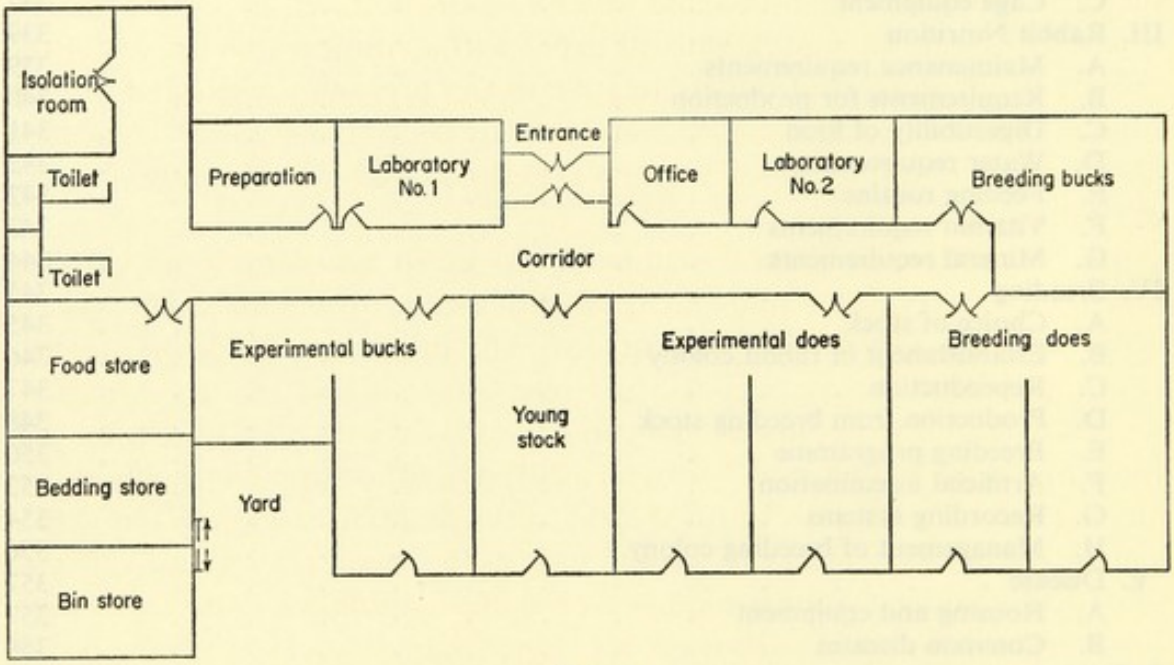

FIG. 1

plan of a rabbit house designed to accommodate breeding and rearing stock as well as adult stock for research purposes.

Breeding does, bucks, young growing stock and adult stock for experimental purposes should be housed in separate rooms. The nutritional requirements and general management of each of these groups of rabbits are different, so that separate housing facilitates good rabbit husbandry. In particular, the breeding does give much better results if given separate accommodation where they receive the minimum disturbance. Each animal room should be treated as a separate unit, with its own access to the outside, and so it should not be necessary to go through one room to get to another.

In effect this means that the rooms should open off a long corridor. A sink and a supply of water to each room is of considerable value, even if drinking water is led to the cages by some form of automatic supply. In conjunction with this, some means of drainage should be employed, either by sloping the floor slightly towards the outside or by having an inside floor drain.

A. VENTILATION

It is just as important to have good ventilation as it is to exclude draughts. The use of timber cages or some other form of solid cage tends to restrict air flow within the building and this fact must be taken into account when designing the ventilation system. If natural ventilation is to be used, windows can be placed above cage height or facing along the gangways between each row of cages. Where cages are constructed round the walls, placing air inlets and outlets can be a problem. If all the inlets and outlets are situated in the walls above cage height, the circulation of air may be insufficient to remove the carbon dioxide, ammonia and water vapour produced by the stock. The efficiency of natural ventilation will depend to a considerable extent on the placing of air inlets and outlets relative to the prevailing wind and airflow round the house. For this reason, natural ventilation cannot be recommended for a laboratory rabbit house where it is desired to have a relatively constant environment.

The alternative to natural ventilation is forced ventilation. Air may be forced into each rabbit room and allowed to escape by windows. This system is probably the nearest to the present ideal. The air pressure inside the building is always slightly above outside atmospheric pressure so that the airflow is always from inside to outside. Air can be fed through a duct system to each room and if desired filters etc., can be incorporated to supply virtually sterile air. Instead of using fans to force air into the building, a suction fan with a similar ducting system can be used to extract the foul air. Fresh air is drawn into the building through windows or baffled air inlets. Apart from the obvious disadvantage that disease organisms may be sucked into the house, it is necessary to take precautions, with the latter system, to prevent the ducting becoming choked with rabbit fur. Using either of these systems of forced ventilation, the number of air changes per hour should be at least four, and it should be possible to increase this rate to approximately ten air changes per hour to cope with hot weather.

An efficient ventilation system, and one which can be simply and rapidly adjusted, is essential in every animal house. Over-ventilation will cause little harm, but may cause an excessive drop in room temperature. Under-ventilation will result in an animal house which is unpleasant to work in because of the high humidity and concentration of foul air. Finally, the risk of disease is far greater in a badly ventilated house, as a build-up of pathogenic bacteria and viruses will rapidly occur in foul stagnant air.

B. HEAT

Rabbits are in general extremely tolerant to a wide variation in temperature. Excessive heat will result in a poor quality pelt, but apart from looking rather untidy the animals will thrive. Although it is not possible to give an ideal temperature for the rabbit house, a temperature of 10-18°C (50-65°F) is desirable for the comfort of those who attend and work with the stock. A room temperature of about 16°C (60°F) can generally be maintained without having to sacrifice good ventilation, so that the relative humidity within the building can be kept in the region of 75%.

The new-born rabbit is particularly susceptible to chilling, and some form of protection must be afforded to the young rabbit for the first two or three weeks of its life. A nest box or tray together with a generous supply of nest building material should be available to the breeding doe if the temperature in the breeding quarters is not controlled to a minimum of 10°C (50°F). If no nest box is provided, the doe must be given plenty of straw, or similar bedding, and allowed to build a nest in the straw. This latter method is of course only of use in solid cages. Providing the doe can build a warm nest for her litter, breeding can be carried on successfully at temperatures of zero and below.

The one occasion when heat is of real value is for bleeding. If this operation has to be carried out inside the animal room, some form of heating which can be directed on to the animal being bled will greatly ease this operation. Similarly, it is advisable to have some form of local heating where new-born or very young animals are weighed or otherwise handled experimentally.

C. LIGHT

Regular inspection of the stock is essential with every form of livestock and this rule is especially important with rabbits. They tend to spend much of their time sitting or lying in the darkest part of the cage, making the early detection of disease difficult. Good natural and artificial lighting is therefore essential.

No firm relationship has been established between light intensity or periodicity and reproductive performance. In fact it is generally believed that day length is unimportant. There do not appear to have been any properly controlled experiments of adequate size on this subject, but the conception rate does drop in the autumn and rise again in the spring. This could of course be attributed to many different factors of which day length is only one. A similar pattern, or rise in spring and fall in autumn, has been observed in animal houses where the temperature has been kept above a minimum of 10°C (50°F). It is therefore unlikely that much of the variation in conception rate can be associated with changes in temperature.

The vitamin D requirement of rabbits is usually satisfied by feeding a vitamin-enriched diet, so there is no need to allow access to direct sunlight.

In fact, the exclusion of direct sunlight is advantageous from the point of view of maintaining the desired temperature in the rabbit house and, perhaps of less importance in laboratory animals, too much sunlight will fade the pelt.

D. RABBIT HOUSE AMENITIES AND EQUIPMENT

1. Isolation facilities

An isolation room is absolutely essential in a laboratory rabbit house. Even where a closed colony is maintained it is occasionally necessary to bring in outside stock for breeding or research purposes and irreparable harm can be done by failing to isolate new stock completely. It should be possible to manage the isolation room as if it was a completely separate unit. Cleaning and other equipment should either be kept in the isolation room itself or in some accommodation other than that normally used for storing equipment. Likewise, a small supply of food can be stored in or near the room and apart from the normal food store. Piped water and washing facilities are an added advantage, though not essential if some such facilities are available close to the isolation room.

2. Laboratory facilities

Where a rabbit colony is housed apart from the main laboratory buildings, a room fitted out as a laboratory should be available for carrying out experimental work on the stock. The size of this room will of course depend on the size of the rabbit colony, the number of research workers and the frequency of stock handling. The room should have ample bench and cupboard space, a sink, hot and cold water, several power points and good lighting.

There are several alternatives to having a laboratory in the rabbit house but each has its disadvantages. If the stock is housed in different rooms as previously suggested, working space at least must be provided in each room even if this means that a trolley is moved from one room to another. Much space which could be used to house more animals is thus wasted. Providing working space in only one animal room has the obvious disadvantage that rabbits have to be taken from one animal room to another, an operation which should be carried out as seldom as possible.

It is never possible to plan routine cleaning and stock management to fit in with research projects, and it is quite impossible to work with rabbits in the same room and at the same time as such routine management tasks are in progress. When all these factors are taken into consideration, the necessity of good working accommodation within the rabbit house becomes obvious.

The above recommendations apply to the situation where several research workers each have occasional use of up to twenty rabbits. If a sufficient number of rabbits is regularly required by the research worker, it may be

preferable to have separate rooms to house each worker's stock; in other words, several laboratories built within the rabbit house with cage accommodation for between fifty and one hundred rabbits in each.

3. Storage and cleaning

a. Food and bedding. Here again the size of store will depend to some extent on the size of the rabbit colony, but there is always the question as to how many rabbits can be kept without having any food store. If a stock of about one hundred adults is kept in one room and there is bin space for 4-5 cwt (200-250 kg) of food in the room, then additional storage space may not be necessary. When the stock is kept in several rooms and breeding is carried on, then storage space in each room is uneconomic. Food and bedding can be purchased more cheaply by the ton, and if a ton of food is consumed in 6-8 weeks this is the best way to buy food. Bedding can usually be stored indefinitely and presents few problems other than the exclusion of vermin.

The food store must be cool, dry, well ventilated and vermin-proof. It should have easy access to a good road for incoming foodstuffs and bedding and be convenient to the animal rooms. Bulk storage of food is becoming increasingly popular with all forms of livestock, but it is unlikely to be of much use in a rabbit colony unless very large numbers of animals are kept. The nutritive requirements of each class of stock will be dealt with later and it is sufficient to point out here that, as breeding stock should be fed differently from growing and adult stock, each ration must be bought in bags and either stored in the bag or transferred to movable storage bins. Any container which has to be filled and emptied from the top should be completely emptied and cleaned before refilling, otherwise a layer of stale food will inevitably accumulate at the foot of the bin. It may be convenient to hold two or three days' supply of food in each rabbit room, in which case circular dustbins of galvanized sheet steel are very useful. Various sizes are available holding up to 2 cwt (100 kg) and they can be easily transported on triangular three-wheeled trolleys. The same type of bin and trolley can be used for transporting bedding (clean or dirty) where solid-floor cages are used. If straw is used for bedding or nest building, a rectangular bin, again mounted on casters, will be found to be more serviceable than a round one. An alternative piece of equipment for transporting bedding is a three-wheeled truck, with removable sides and either solid or inflatable rubber tyres. It is very useful for moving not only foodstuffs and bedding, but also the stock, cages and other equipment.

b. Equipment. The number of rabbits retained in a colony for breeding and experimental purposes may vary considerably from time to time so that there will, on occasions, be a large number of food and water containers not in use. Similarly, if wire cages are used these have to be dismantled for cleaning and if not required are better stored in their sections. Storage accommodation for spare equipment is too often overlooked when planning the layout of a

rabbit house, and this leads to the neglect and rapid deterioration of equipment.

The design and construction of the rabbit house should be such that routine cleaning and disinfecting are easily carried out. The floors and walls should be surfaced with some impervious material which can be sprayed or scrubbed. A considerable amount of rabbit fur will collect on all surfaces inside the room, especially rough surfaces, and routine cleaning of the whole rabbit house is essential. Timber or other forms of solid cages may be cleaned and disinfected *in situ*, but this cannot be done satisfactorily with wire cages. In either case it is much better to remove empty cages from the rabbit house for thorough cleaning. Small equipment, such as food and water troughs, can either be washed and sterilized in each rabbit room or in a separate washing-up room fitted with special cleaning equipment. If one cleaning room is decided upon it should be so designed that there is a one-way movement of equipment; dirty in at one end and clean out at the other. The same door should not be used for taking out the clean equipment as is used for taking in the dirty.

The movement of stock and light equipment is more or less a continuous operation in a rabbit colony and for this purpose light-weight metal trolleys are invaluable. A trolley to carry cage-cleaning equipment and on which the rabbit can be placed while its cage is being cleaned is very useful. A rabbit does not like standing on a slippery surface and will not remain on a smooth trolley top if it can possibly get off. This difficulty can be overcome by spreading a piece of sacking or rough canvas inside a shallow metal tray; the rabbit can then be placed on the tray. This piece of equipment can be easily disinfected between each animal if necessary. A shallow tray measuring about 18×12 in (45×30 cm) is useful for holding rabbits for either routine or experimental purposes. It tends to prevent the animal from wandering about the trolley or bench top and therefore lessens the risk of equipment and record books being damaged.

4. Travelling boxes

The following points should be taken into consideration when designing a rabbit travelling box.

a. The size of container relative to the size of the rabbit. Breeds which have an average weight of over 5 kg require a container $18 \times 12 \times 12$ in ($45 \times 30 \times 30$ cm) high, while a container $15 \times 9 \times 12$ in ($38 \times 23 \times 30$ cm) high is suitable for medium and small breeds.

b. The container may be constructed of wood or fibreboard, but in either case it should be sufficiently waterproof to avoid the escape of urine.

c. It should be possible for air to enter the container from the top and from the top quarter of each side. Air inlets made below this level in the sides

are liable to cause draughts. There should be half-inch (1·2 cm) thick rails along the bottom of the container to keep it off the ground; and there should be similar rails along the top and at least two of the sides so that ventilation is not restricted when a number of containers are stacked together.

II. Cages

A rapid expansion in the commercial rabbit-meat industry in Britain between 1958 and 1960 led to an equally rapid expansion in ancillary trades. A large variety of rabbit cages became available, many of these designs were based on traditional specifications while others were more revolutionary. As one might expect, the cost of the various types now on the market bears little relationship to their utility, so that each design must be judged on its merits.

Basically, rabbit cages can be divided into three main categories, namely solid, wire, and a combination of the two.

A. SOLID CAGES

Solid cages can be subdivided into those which the rabbit will eat and those which the rabbit will not eat. This may seem rather a strange classification of cage material but it is a practical one.

Cages made from chipboard as shown in Fig. 2 (*a*, *b*, *c*) are among the cheapest to make. They are based on a timber cage designed by Wilson (1935) but incorporate several modifications. Each unit of six cages is made up of sections of chipboard which can be replaced if necessary or repaired with a mixture of coarse sawdust and bonding compound. The litter boards attached to the woven mesh doors are chipboard off-cuts. They are the only prominent wooden surfaces and it has been found that the rabbit will tend to gnaw this convenient and easily replaceable fitting rather than the walls of the hutch. Each cage is 2 ft × 2 ft × 1 ft 9 in (60 × 60 × 53 cm) and is quite satisfactory for animals weighing 2-3 kg mature weight. The centre partition is withdrawn to convert two cages into one breeding cage. Straw is used as litter and for the doe to make a nest. Nest boxes and partitions have been used but were not found to give a higher weaning percentage. The provision of nest boxes may be advantageous if the strain of rabbits is nervous; otherwise perfectly satisfactory results can be obtained, even in an unheated building, by letting the doe build her nest in the straw. If the rabbits are not handled frequently, one door for each half-unit of three cages can be constructed from timber and 17 s.w.g. wire netting. However, one door for each cage has been found to be preferable under laboratory conditions where the rabbits are handled frequently and it is important to prevent one animal getting into another's cage.

FIG. 2(*a*)

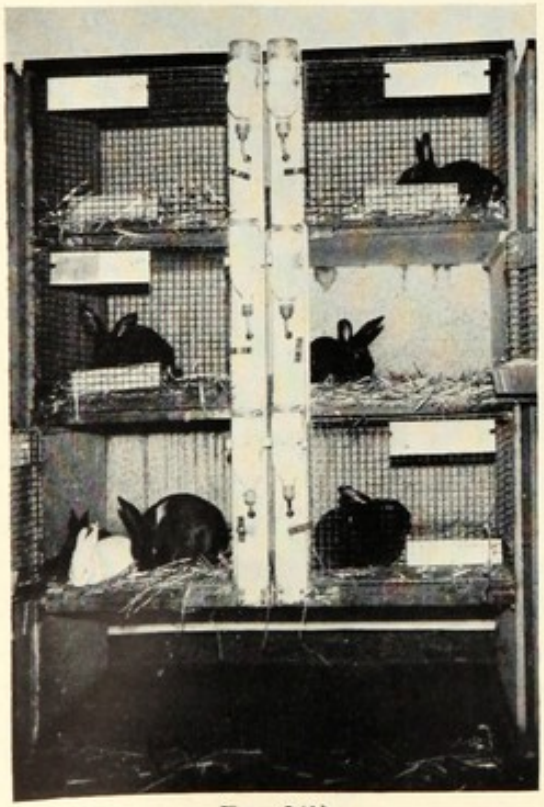

FIG. 2(*b*)

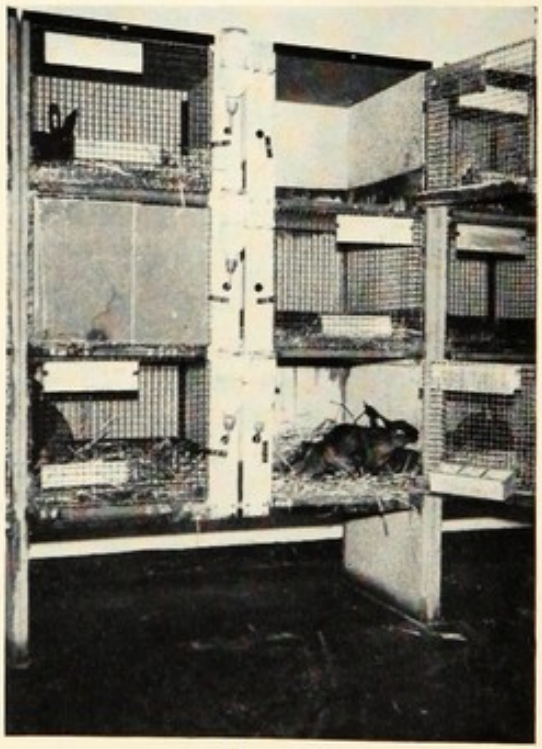

FIG. 2(*c*)

Figure 3 shows an alternative type of solid cage. The floor is of soft timber and the walls are of 26 s.w.g. galvanized sheet steel mounted on a $1\frac{1}{2} \times 1$ in ($3{\cdot}8 \times 2{\cdot}5$ cm) rough-cut timber frame. The cage is $5 \times 3 \times 2$ ft ($150 \times 90 \times 60$ cm) and it is used for breeding does weighing up to 8 kg. Each cage is a separate unit and one is nested on top of another so that only the door need be taken off to remove any one cage. As in the cages described above, straw is used as bedding and for the doe to build her nest. Bedding is added as required and the cages are cleaned every two to three months or after a litter

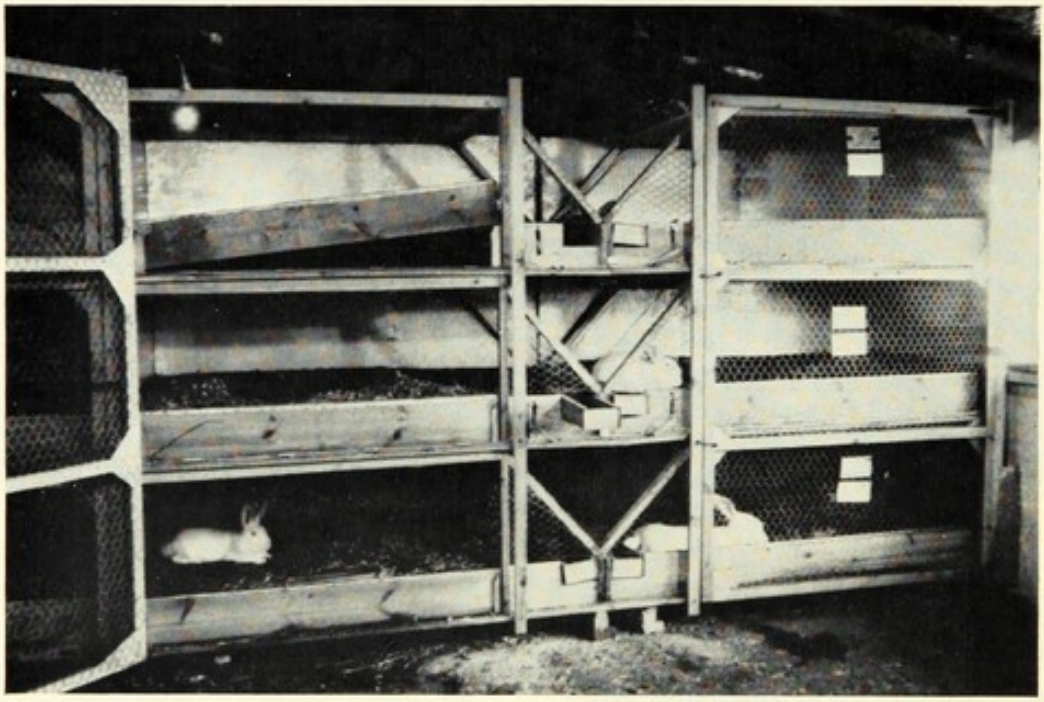

FIG. 3

is weaned. The only part of this cage which can be gnawed by the rabbit is the litter board and this can be protected with folded sheet steel. Trouble has never been experienced with rabbits gnawing the timber floor which is well covered with bedding.

Turning now to solid cages with part wire floors, the wire part is usually a $\frac{3}{4} \times \frac{3}{4}$ in ($1{\cdot}9 \times 1{\cdot}9$ cm) woven mesh using 12 to 14 s.w.g. galvanized (and sometimes plastic coated) steel wire. A plastic coated or galvanized steel tray is placed under the wire floor to catch the urine and droppings. A partition separates the solid from the wire floor and a hole cut in the partition, 6 in (15 cm) above floor level, permits access between the compartments. The doe kindles on the solid floor and the partition prevents the young rabbits leaving the nest until they are nearly three weeks of age. This type of cage has the disadvantages of both solid and wire floors; it is therefore not recommended. Similarly, a cage which has solid timber walls and a wire floor still has the disadvantage that the rabbit will eat the timber parts. If individual droppings

trays are placed under the wire floors, this type of cage can be used outside or in an unheated house, but if automatic cleaning is used draughts entering the cage *via* the floor can cause trouble.

B. WIRE CAGES

Wire battery cages constructed on the same principle as poultry battery cages have been in use in the U.S.A. for many years, for both breeding and rearing stock. It is only in the last few years that this type of cage has gained in popularity in Great Britain. As far as cost goes, an all-metal sectional battery cage of good quality is by far the most expensive method of housing rabbits. Figure 4(*a*) shows one of these battery cage units. The side and back walls are of galvanized sheet steel partitions which are removable. A cage measuring $24 \times 22 \times 15$ in ($60 \times 55 \times 38$ cm) is the minimum size of unit. A breeding cage as shown in Fig. 4(*b*) is $48 \times 22 \times 15$ in ($120 \times 55 \times 38$ cm). Nest

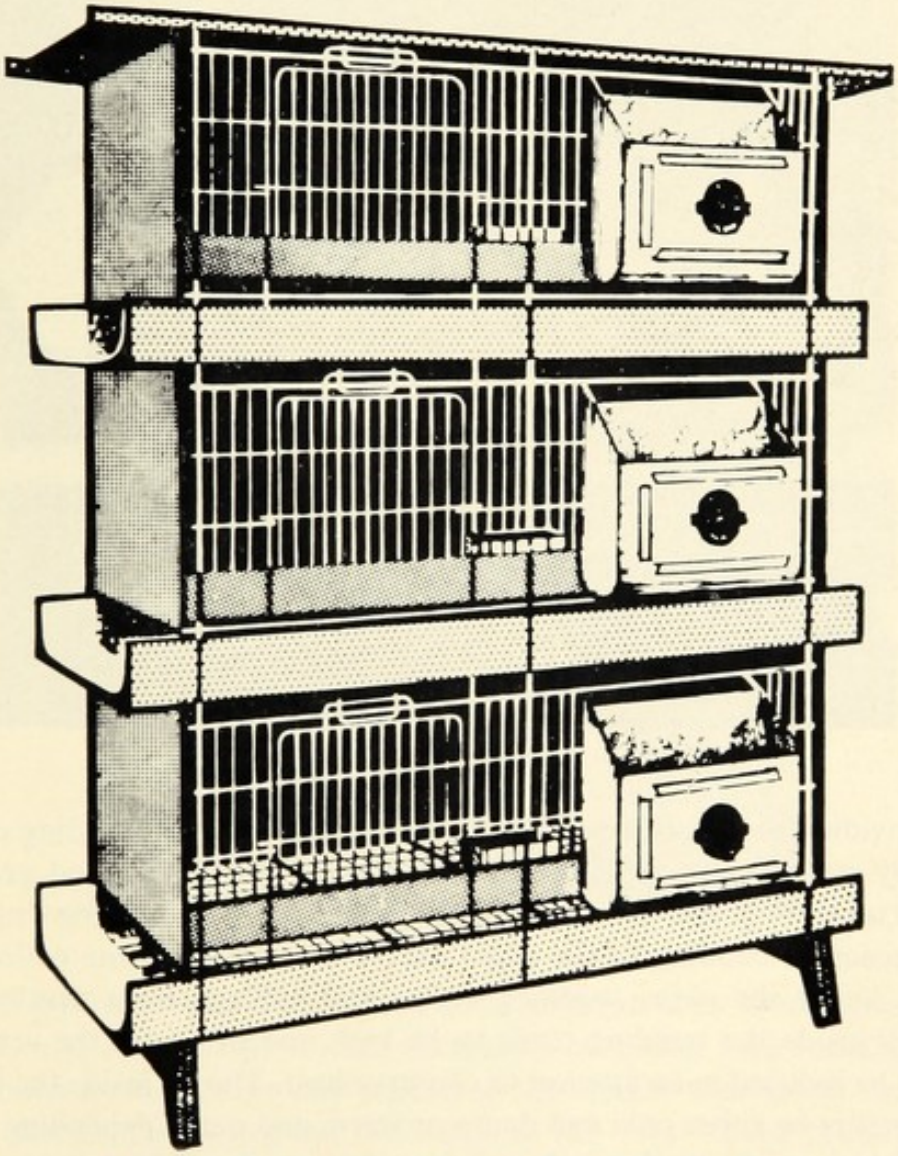

FIG. 4(*a*). (By courtesy of Cope & Cope Ltd., Reading)

boxes or trays, together with some form of litter, must be given to breeding does for at least three weeks after the litter is born and it is advisable to put the nest box into the cage at least one week before the litter is due. Perfectly satisfactory results can be obtained with breeding stock kept exclusively on

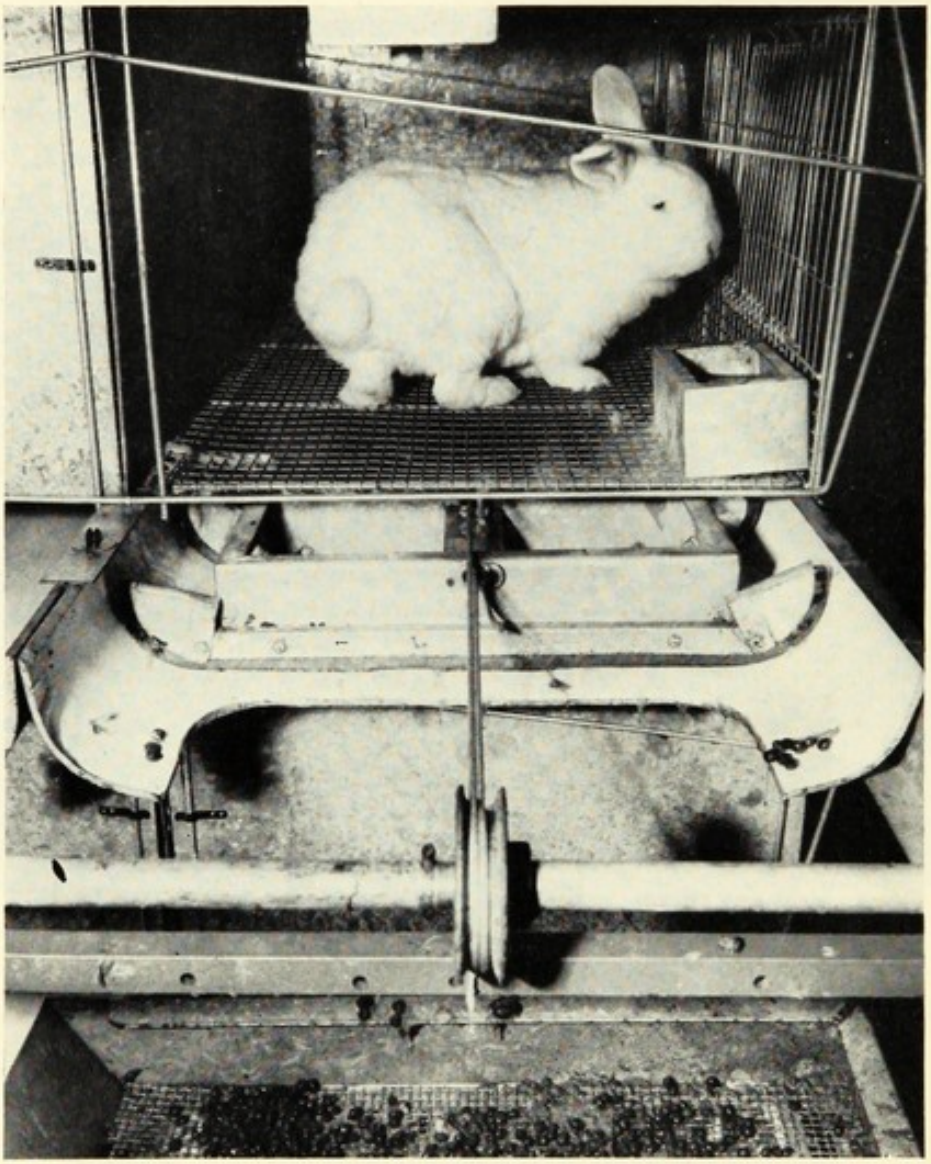

FIG. 4(*b*)

wire providing the management is of a high standard. The building in which a battery system is installed should have an insulated roof and great care must be taken to conserve heat without restricting ventilation too drastically. The absence of bedding in the cages means that all the urine collects in a channel under the cages together with water spillage from drinkers. The humidity inside the building tends to be high and in winter the ventilation tends to be reduced in an attempt to conserve heat. The air inside the building will therefore be either cold and damp or warm and damp depending on how successful heat conservation is. In either case the stock will become susceptible

to respiratory disease which may develop into pneumonia. A rabbit house which is insulated, heated and has power-operated ventilation is the ideal for a battery cage installation.

FIG. 5(*a*)

Wire cages have been used to a considerable extent by commercial rabbit breeders, who are usually working with stock weighing 5 kg and above, and it is perhaps not surprising that rabbits of this weight tend to get sore hocks on wire floors. Increasing the thickness of the wire would probably help considerably in avoiding this complaint, but using heavier gauge wire adds

appreciably to the cost of the floor. An alternative is to use a plastic-covered wire floor, but this is just as expensive. Where the adult weight of the stock is under 5 kg, sore hocks will be less frequent.

Some breeders prefer to keep their breeding stock in solid-floor cages and

FIG. 5(*b*)

their rearing stock in wire cages. Those who advocate this system claim that breeding performance, especially in the winter months, is better on solid floors. This might well be true, but the reason could be attributable to an unsuitable house for the wire cages.

A battery cage unit can be most economically employed where the cages can be erected back to back and with mechanical cleaning and automatic watering. The longer the unit the better, otherwise the cost of the mechanical cleaning equipment for a number of short units will make the total cost prohibitive. If this type of cage is to be used it is almost essential to build a

house to suit the cage rather than attempt to fit the cages into an existing building, especially where the different classes of stock are housed separately. It is certainly not worth sacrificing isolation of breeding and rearing stock in favour of a battery cage system. If the cages can be satisfactorily incorporated into a laboratory rabbit colony consisting mainly of non-breeding stock of light to medium weight, then this system of rabbit housing has much to commend it.

Figure 5 (*a*, *b*) shows the battery cage in use at the National Institute for Medical Research, Mill Hill, London (Short, 1960). The cages are constructed of 10 s.w.g. wire and measure 17 × 19 × 13 in (43 × 48 × 33 cm). Feeding and watering is on the cafeteria system. This type of battery has proved very satisfactory for rearing stock and may profitably be adapted for housing breeding stock.

Whatever type of cage is employed, the layout of the cages in each room requires careful consideration. As previously mentioned, wire cages are most economic when mounted three tiers high and back to back. A gangway is therefore necessary down each side of the bank of cages. This obviously wastes more floor space than having the cages face to face with one gangway between them. If solid cages are placed face to face, and again three tiers high, a gap of at least 6 in (15 cm) and preferably 1 ft (30 cm) should be left between the back of the cages and the wall to allow free circulation of air behind the cages.

C. CAGE EQUIPMENT

1. Nest boxes

It has already been pointed out that nest boxes are necessary only in wire cages. They may, however, be used in solid cages where sawdust, shavings, or some other similar type of bedding is used instead of straw.

Providing nest boxes not only increases the cost of housing for each breeding doe, but also entails a considerable amount of extra work. The nest box is liable to be gnawed by the doe, and it is difficult to clean and disinfect properly. For these reasons there is much to be said for using fibreboard nest boxes which can be destroyed after the litter leaves the nest. If the breeding quarters are not too cold, nest trays are a satisfactory alternative. These may also be made of fibreboard and are undoubtedly the cheapest form of container for the doe to kindle in.

The size of the nest box or tray will depend on the size of the doe and the average litter size of the strain of rabbits. The area of either a box or a tray should be approximately 1½-2 ft² (0·15-0·2 m²) for does weighing 3 to 5 kg and the height of a box should be at least 9 in (23 cm).

2. Food troughs

Heavy glazed earthenware pots are generally recommended as being suitable for feeding rabbits. They have a number of disadvantages however, and the most important of these is the ease with which they can be broken during

cleaning. Even the heaviest of these pots can be overturned if the rabbit is so minded, and although this rarely breaks them, they do tend to crack. Cleaning of earthwenare pots has to be done carefully, otherwise cracks will soon appear and cracked pots cannot be sterilized properly and should never be used. Finally, the pots are quite expensive and do not permit a large number of animals to feed at once. In fact, all too frequently one rabbit will sit in such a dish and prevent any of the others from eating.

There are available on the market several types of galvanized steel troughs commonly used for feeding and watering poultry. The cost of these troughs is little more than the cost of earthenware pots, and they will last infinitely longer and accommodate a larger number of feeding rabbits at one time. If this type of trough is used, it can be either hooked on to the door of the cage, as is shown in Fig. 2, or fitted into the cage like a drawer, as in Fig. 3.

Bulk hoppers for pelleted food are very useful in certain cases, especially for young rearing stock being fed *ad lib.*, but it is difficult to see when the hopper is empty or in need of cleaning out, particularly with hoppers attached to the top tier of a three-tier cage unit.

The feeding of green food and hay is becoming less common in laboratory rabbit colonies so that hay racks are generally not required.

If it is desirable to have hay racks fitted to the cages, this can be done as shown in Fig. 3, or, if the cage unit has some form of wire door, the hay rack can be fitted to the outside of the door. Much time can be saved if all the feeding can be done without opening the cage door.

3. Watering

A satisfactory watering system is probably one of the most difficult problems to solve in relation to equipment. Open troughs, similar to those described for feeding, can be used, but they are too easily filled with bedding and require frequent cleaning. Glass or plastic bottles fitted with corks or rubber bungs and brass tubing, as shown in Fig. 2(*a*), are quite satisfactory, but filling these bottles is a time-consuming job. The obvious requirement is an automatic water system. Unfortunately none of the systems available at present is completely trouble free. In any system which employs valves, the valves are liable to stick and either the cage is flooded with water or the rabbit gets no water. (The flooding is less damaging in wire cages.) Some valves, even when operating correctly, restrict water flow to such an extent that the rabbit does not get sufficient.

A system which appears to give relatively little trouble, and is probably the simplest of all, depends on the principle of gravity flow. A pressure reduction tank is mounted at one end of each tier of cages and the water level in this tank is accurately maintained by a float valve. A horizontal pipe is led from the tank in front of each cage in that particular tier and a vertical pipe is led into each cage from junctions in the horizontal pipe. The water level in the

pressure reduction tank is adjusted so that the water level in the vertical tubes is just below their open end. The rabbit drinks by sucking the water from the end of the vertical tube. A modification of this system is to have the vertical tube opening out into a small drinking bowl. The level of water is then adjusted to the required depth in the bowl.

III. Rabbit Nutrition

The aim with laboratory stocks of rabbits is usually slightly different from the aim of the commercial rabbit breeder. Standardization of nutrition and other environmental factors is essential to the interpretation of results of experiments with all forms of livestock, and the rabbit is no exception to this rule. The commercial breeder, on the other hand, will change the rations he feeds, to obtain better results, as often as he thinks necessary. The fact that laboratory stocks should be fed a standard diet immediately limits the choice of ration to cereals and concentrate mixtures. The most satisfactory ration is undoubtedly a manufactured rabbit pellet, of which there are many on the market, and several will give satisfactory results. Unfortunately there are still many variables in proprietary rations, which are liable to cause trouble. For instance, the country of origin of the various constituents and even the constituents themselves can and do change continuously in relation to world food prices, and although these changes in the ration do not appreciably affect the starch or protein equivalent, they may cause dietary upsets in the stock. Rabbits are certainly very susceptible to nutritional upsets, perhaps through our lack of knowledge of their nutritional requirements.

A. MAINTENANCE REQUIREMENTS

Part of the ration fed to any animal is utilized to maintain its state of equilibrium. In this state, the animal neither loses nor gains weight but uses

Table I

Theoretical maintenance requirements for rabbits

Body wt kg	Theoretical maintenance requirements Cals/rabbit/day	g digestible protein per rabbit/day
1·0	124	5·2
1·5	164	6·5
2·0	202	7·8
2·5	242	8·8
3·0	282	9·9
3·5	322	10·9
4·0	360	11·9
4·5	400	12·9
5·0	438	14·0

the energy derived from this part of the ration to fulfil all of its vital functions. The amount of energy required to keep an adult rabbit in this state of equilibrium is called the basal metabolic rate. The maintenance requirement (which is obtained by taking twice the estimated basal metabolic rate) for adult rabbits has been investigated by Lee (1939a) and Axelsson (1949a, b) The approximate theoretical maintenance requirements for different sizes of rabbits is given in Table I.

A certain amount of digestible protein is also necessary in the maintenance ration; the approximate quantity is also given in Table I.

B. REQUIREMENTS FOR PRODUCTION

It may be necessary in a laboratory stock of rabbits to hold a number of adult animals for some time until they are required for experimental purposes. In such a case the maintenance requirements given in Table I should approximate to the total requirements of these animals. As soon as the animals are used, whether for breeding or experimental purposes, the ration must be increased in proportion to the energy expended by the rabbit. Similarly, the young growing animal which is building a considerable amount of muscle tissue requires an addition to the maintenance ration.

1. Pregnant does

In the latter part of gestation the doe requires additional energy, protein, vitamins and minerals. Provided the breeding ration contains a vitamin and mineral supplement, increasing the amount of the ration fed will amply satisfy the vitamin and mineral requirement. The breeding ration should, however, contain a slightly higher proportion of protein than the maintenance ration. Diet SG1, a ration developed at the National Institute for Medical Research (Short and Gammage, 1959), suggests that 14·7% protein is sufficient, while the Rockland (U.S.A.) Rabbit Ration suggests 18·6%. The theoretical composition of these two rations is given in Table II below, together with the composition suggested by Templeton (1939) and that suggested by the Rabbit Experiment Station, Fontana (U.S.A.).

TABLE II

Composition of rabbit rations

	Diet SG1	Rockland rabbit ration	Theoretical requirements recommended[1]	Fontana ration
Digestible crude protein	22·3%	18·6%	16-20%	20·8%
Ether extract	3·4%	2·6%	3-5·5%	3·7%
Nitrogen-free extract	36·6%	61·7%	40-44%	c. 45%
Crude fibre	4·5%	13·7%	14-20%	15·1%
Ash	7·3%	6·5%	4·5-6·5%	6·8%

[1] Templeton, 1939.

2. Growing stock

A ration very similar to that required by pregnant and lactating does is also required by growing stock. The energy and protein requirements of the young rabbit have been investigated by Templeton and Kellog (1959), and the amount of protein and number of calories required to increase the animal's live weight by one pound is given in Table III.

TABLE III

Nutritional requirements of the growing rabbit

Age in weeks	Approx wt lbs	Approx gain in wt oz/wk	Cals required per lb live wt gain	g digestible crude protein per lb live wt gain
8	2·0	5·0	1,770	85
10	2·6	5·5	2,136	109
12	3·3	5·5	2,500	123
14	4·0	4·0	2,860	138
16	4·5	3·5	3,236	149
18	5·0	3·0	3,590	160
20	5·4	2·5	3,955	170
22	5·7	2·0	4,318	180
24	6·0	1·5	4,680	190

3. Breeding males

Bucks which are used regularly for natural mating or artificial insemination require only slightly more energy and protein than for maintenance. Care should be taken not to overfeed working bucks, particularly those of the heavier strains of rabbits; *ad lib.* feeding is therefore not recommended. Failure of the buck to work can very often be attributed to the animal becoming too fat.

C. DIGESTIBILITY OF FOOD

The only way in which the digestibility of foodstuff can be determined is by direct experimentation with the food and animal in question. The digestibility of a particular foodstuff as determined experimentally with sheep or cattle will be quite different from the digestibility of the same food when tested on the rabbit. Care should therefore be taken when compiling a ration for rabbits not to use digestibility values from tables prepared from data on ruminants.

The amount of fibre in the diet will affect the digestibility of the ration as a whole and especially the digestibility of protein. The higher the fibre content, the lower will be the proportion of protein digested. There have been many experiments on the digestibility of feeding-stuffs in the rabbit and most of this work has been reviewed by Jarl (1944).

Another factor to be considered in relation to the digestibility of food is the rabbits' habit of coprophagy. The coprophagous pellets are taken from the anus and reingested. It is thought that this habit enables the rabbit to obtain certain of the B complex vitamins which are present in the pellets following the synthesis of this vitamin in the caecum and large intestine by the normal microbial flora (Baker, 1944).

D. WATER REQUIREMENTS

It has been shown by Wilson and Morris (1932) that approximately two-thirds of the adult rabbit is water, and Hill (1920) has shown that rabbits kept in direct sunlight will loose up to 23 g of water per hour compared with 2 or 3 g per hour when the animals are kept in the shade. This indicates that although the water requirement is extremely variable some source of water must be made available in the diet. Lactating does will require considerably more water than growing or non-breeding adult stock, the actual intake depending on the milk production of the doe. Withholding water is liable to cause vices such as urine drinking and cannibalism (Wilson 1943b), and even when succulent food forms part of the ration, water should always be available (Kennaway, 1943).

There are many commercial rabbit farms in Great Britain and on the Continent where water is not supplied as a routine practice. The feeding of succulent green food and roots is said to satisfy the rabbit's water requirements. Water is, however, made available to the stock in lengthy dry spells.

In one particular feeding trial carried out in cold weather, the water consumption of rabbits being fed a concentrate pellet was approximately one pint (570 ml) per animal per day. (The rabbits weighed from 5-7 kg.) When fresh-cut or one-day wilted lucerne was added *ad lib.* to the ration, the water consumption of this group dropped markedly. Some animals took no water while others still took up to about half a pint (285 ml) per day.

It has already been stated that a concentrate pellet is the most convenient food for laboratory populations of rabbits. In these circumstances it is absolutely essential that a supply of fresh clean drinking water should be available to all classes of stock.

E. FEEDING ROUTINE

In theory, *ad lib.* feeding of rabbits is desirable both because the rabbit is a continuous feeder and because it saves labour. Experience with a number of strains of rabbits varying in weight from 1 to 8 kg suggests that rationing the diet is by far the safest procedure with breeding stock and potential breeding stock. In some strains of rabbits all classes of stock can be fed a concentrate ration *ad lib.* without any deleterious affects on breeding performance. One particular strain weighing approximately 2 kg was fed a low fibre concentrate

ration *ad lib.* and, excluding lactating does, the consumption of pellets was between 4 and 5 oz (113 and 142 g) per animal per day. It should be added, however, that these animals were bedded down on good quality oat straw. Presumably the rabbits satisfied their bulk requirement by eating the straw. The average quantity of straw consumed per animal per day was not recorded, but it was certainly quite considerable.

Little trouble has been experienced with the various strains, in the weight range mentioned above, when feeding growing stock *ad lib.*; but when a strain reaches sexual maturity it has been found better to restrict the diet. In the latter part of pregnancy and during lactation the doe can be fed *ad lib.*, but if this practice is continued after weaning the litter, difficulty may be experienced in getting the doe mated again.

F. VITAMIN REQUIREMENTS

In 1906 Hopkins showed that vitamins were essential to life, and since that time much work has been done to determine the vitamin requirements of all classes of livestock. The chemical composition of vitamins has also been widely investigated with the result that many can now be synthesized. The addition of synthetic vitamins is therefore a simple and convenient method of ensuring that an adequate supply of vitamins is present in the ration.

1. Vitamin A

Much work has been done on the effects of a deficiency of vitamin A or its precursor carotene and this work has been amply reviewed by Sandford *et al.* (1957). A theoretical requirement of 4 mg vitamin A or 25 mg B carotene per kg body weight has been suggested by Coward (1953) as being sufficient for all classes of livestock.

The likely sources of this vitamin in a pelleted ration are dried yellow maize and fish oils. Vitamin A may also be added to the ration in synthetic form. By coating minute quantities of the vitamins with crystalline wax or gelatine and including these particles in the ration, the vitamin remains stable for much longer. Rations which have been stored for two or three months, or in sub-optimum conditions, are thus less likely to be deficient in vitamin A. Grass meal may also be comparatively rich in carotene, but the carotene content is found to vary widely with the quality of the meal.

2. Vitamin B complex

It has already been mentioned that some of the vitamins in this group are supplied indirectly by bacterial synthesis in the gut. The coprophagous habit of the rabbit prevents deficiency of most of the vitamin B group and apart from coprophagy the rabbit will obtain vitamins of the B complex from the aleurone layer of cereal grains.

3. Vitamin C

There is unlikely to be a deficiency of this vitamin, as it is synthesized by the rabbit. There is therefore no need to add synthetic vitamin C to the ration even when green food and other sources are absent.

4. Vitamin D

It has been reported by Jarl (1948) that rabbits do not suffer from vitamin D deficiency and that although calcification may be improved by adding D_3 to the ration, growth rate was not depressed by normal rations. If vitamin D is added, the synthetic form is the most convenient and it is incorporated in the ration in the same way as vitamin A. It has been shown that excess vitamin D may cause deposition of calcium salts in various organs, and haemorrhagic lesions of the intestine (Innes, 1931; Okushima, 1937; Orzechowski and Schreiber, 1943).

5. Vitamin E

As the normal rabbit ration contains cereal grains, vitamin E is unlikely to be deficient in the diet. Excess cod-liver oil may cause a deficiency of vitamin E. Unlike its affect on other animals, this deficiency does not affect fertility in the rabbit but causes muscular dystrophy. (A number of references to work in this field is given by Sandford *et al.*, 1957.) The suggested vitamin E requirement per day has been given as 1·0 mg per kg body weight (Mackenzie and McCollum, 1940) while Eppstein and Morgulis (1941) suggest that 0·32 mg of tocopherol per kg body weight is sufficient.

G. MINERAL REQUIREMENTS

1. Calcium and phosphorus

The calcium requirement of the rabbit may be satisfied by a supply of organic or inorganic calcium compounds depending on the diet. In either case calcium will reach the blood in a simple form. The small quantity present in the blood is under hormone control and is related to blood-clotting time and to heart contractions. Most of the calcium in the body is found in the bones and teeth, and the relative proportions of calcium, phosphorus and vitamin D are of importance in determining the quality of these tissues. Unlike calcium, phosphorus is more uniformly distributed through the body, mainly in the form of phosphoproteins.

2. Sodium and potassium

As with calcium and phosphorus, there is a balance between sodium and potassium which is as important as the absolute quantity of each. The body consists of about 0·1% sodium, most of which is in the blood. Excess sodium chloride in the ration will result in the rabbit drinking an excessive amount of

water, while excess potassium salts will be rapidly removed from the body in the urine.

Sodium chloride should be present in the ration to a level of about 0·5%. It has been suggested by Templeton (1938) that salt-licks be available to breeding does, but where a properly balanced pelleted ration is fed, this should not be necessary.

3. Iodine

The iodine content of the body is less than one part per million and most of this element is present in the thyroid gland.

It has been shown by Stewart and Menne (1953) that iodine is related to thyroid activity and metabolic rate in the rabbit. It is unlikely, however, that this element will be deficient in the diet.

IV. Breeding

A. CHOICE OF STOCK

There is a large number of breeds of rabbits which, in the main, have been produced by selecting visible mutants. Very few breeds have been selected for characters other than coat characters and conformation, and those breeds which have been developed for commercial meat production are in general not suitable for the research worker.

The breed or breeds of rabbits to be maintained in a laboratory colony should be related to the type of research or routine use of the stock. There is no point in keeping a large strain of rabbits where a smaller one will do, and similarly there is little point in keeping too small a breed such as the Netherland Dwarf which is difficult to manage and is not prolific. It is far more important to obtain and develop a good strain within a breed than to pay attention to hypothetical breed standards.

The research worker who is interested in obtaining a regular supply of blood from his rabbits has probably more factors to consider in choosing his stock than any other. Although size is of obvious importance, this character should be considered in relation to the breeding performance of the strain. There are available strains of White and Brown Lop rabbits, of Danish origin, which have a mature body weight of over 7 kg. These animals will give a copious supply of blood, but unless they are strictly rationed and carefully managed, considerable difficulty will be experienced in breeding these strains. Another character which makes Lop rabbits suitable for bleeding is their large ear size and the consequent ease with which the marginal ear vein can be manipulated. An albino animal is an additional advantage, but an otherwise suitable strain should not be discarded just because it is coloured. If such a

pure strain of Lop rabbits is chosen, it cannot be over-emphasized that the breeding stock should be strictly rationed and mated regularly, otherwise the conception rate is likely to be low. In laboratory stocks it may be necessary to breed according to a production plan, which in turn is geared to a research programme. To meet these requirements it may be necessary to hold unmated breeding stock for several weeks or months. Strains of large Lop rabbits would be most unsuitable in those conditions. An alternative would be to use a good strain of New Zealand White or preferably Danish Landrace doe and cross with a White Lop buck. The offspring will have most of the advantages of the Lop breed but will of course be intermediate in size. The actual size will depend on the body size of the strains used in the cross but should be in the region of 5 kg. The one disadvantage with this system is that a supply of White Lop bucks for crossing must be ensured by either maintaining a small colony of pure White Lops or by buying in replacement bucks. As neither of these programmes is without difficulties it may be decided to use only the one importation of White Lop bucks, and thereafter to breed the females pure and the stud males from half-Lop progeny. A certain amount of selection can be carried out to maintain body size and ear size in breeding males.

Where rabbits are used in other specialized forms of research, involving for example skin tests, a breed such as the New Zealand Red is particularly suitable. For most diagnostic and research purposes, however, a medium sized strain having a mature body weight within the range 2 to 4 kg is the obvious choice.

Little useful purpose is served in describing the numerous breeds of rabbits as the variation within each is so large. A summary of the characteristics of the common breeds is tabulated in the *UFAW Handbook* (1957) and more detailed information on the genetics of the many breeds of rabbits has been compiled by Robinson (1958).

B. ESTABLISHMENT OF RABBIT COLONY

It has already been stated that choice of strain within a breed is more important than choice of breed. The number of different strains maintained will depend mainly on the number of cages available for housing breeding stock and young replacement breeding stock. Approximately one hundred cages are required per strain if the strain is closed to all outside blood. With this number of cages it is possible to preserve a relatively constant genetic constitution within the strain from one generation to the next. As an alternative it would be possible to apply some mild selection to the strain with a view to improving its breeding performance. From the research worker's point of view it is desirable to have a continuous supply of genetically uniform animals for experimentation. It is just as important to know whether the

results of experiments carried out on one strain of rabbits are repeatable when a different strain is used. For this reason it is desirable to maintain as many different strains as facilities will permit rather than to devote the entire facilities to one strain. This point will be considered again in relation to the breeding programme.

The development of inbred strains of rabbits is beyond the facilities of most rabbit colonies. It cannot be stated with any degree of certainty how many lines must be started on a full sib-mating programme to obtain one line which will survive for over twenty generations of inbreeding; probably in the region of twenty. The majority of lines will be lost after the first few generations of inbreeding. After the inbreeding coefficient has reached 60% there is less chance of losing the line, and by the time twenty generations of full sib-mating have been completed the theoretical genetic variation remaining in the line is 1·4% of that present when the line was started. Although an inbred line is genetically uniform, it is usually more susceptible to changes in the environment, and the observed or phenotypic variation in an inbred line may be considerable. To reduce the environmental variation to a minimum, a uniform and high standard of management is necessary if inbred lines are to be kept successfully. Unless there is some particular line of research being followed which requires inbred rabbits, economic factors alone make it advisable to establish a colony consisting of one or more strains in which the rate of inbreeding is kept at a minimum. (See also Ch. 8.)

C. REPRODUCTION

The age at which rabbits are sexually mature depends to a considerable extent on the mature body weight of the strain. It is possible to mate bucks and does of strains weighing under 3 kg at 4 to 5 months of age, while at the other extreme rabbits from strains which have an average body weight of over 6 kg cannot usually be mated until the does are 8 months old and the bucks from 6 to 7 months old. Poor management, disease, a low plane of nutrition or any other stress factors will of course retard sexual maturity quite appreciably. Although it is often stated that there is no definite oestrous cycle in the rabbit, it now seems likely that there may be. It has been suggested by Templeton and Kellog (1959) that there is a 15-16 day oestrous cycle and that conception can occur between day 2 and day 14 of this cycle. There are of course short periods of anoestrus while follicles are maturing and these periods appear to be much longer in winter than in spring and summer. Even when it is possible to get does mated in winter, the conception rate (as judged by the doe producing a litter) is considerably lower than in spring and summer.

There is no reliable method of telling whether or not a doe will take the buck, and while it is true that if the doe's vulva is swollen and moist she will

usually mate, the absence of this sign does not necessarily mean that she will not mate. The most satisfactory technique for mating does in an experimental unit where accurate records are necessary, is to take the doe to the buck's cage and observe the mating. A buck and doe which are in good breeding condition will complete coitus within minutes of the doe being introduced to the buck. Wherever possible the doe should be taken to the buck and not *vice versa* as the buck is less likely to mate in a strange cage and the doe is more likely to fight the buck in her own cage. A regular attendant should supervise the matings, as a stranger handling the animals, or any other disturbance, is liable to put the animals off mating.

Ovulation occurs approximately ten hours after mating and any sexual stimulus to a doe which has ripe follicles is likely to cause the shedding of those follicles. The mounting of one doe by another may cause ovulation and in such an instance, where ovulation is induced and fertilization does not follow, the doe will become pseudopregnant; the corpora lutea will develop and function for some time despite the fact that the ova have not been fertilized. Pseudopregnancy usually lasts 17 to 19 days and towards the end of this time there may be a certain amount of mammary development and the doe may build a nest. Ovulation is suppressed during pseudopregnancy and mating cannot be successfully carried out until immediately after pseudopregnancy has ended, about 18 to 20 days after its onset. This fact makes it advisable to house all breeding does individually for at least three weeks prior to mating them.

Gestation in the rabbit is approximately 31 days, but it is quite possible to have a viable litter from 29 to 34 days after mating.

D. PRODUCTION FROM BREEDING STOCK

Whether laboratory breeding stock is maintained to produce animals for research or as the subject of research, the object is usually to obtain the maximum output per doe. There is virtually no limit to the number of does that can be artificially inseminated with spermatozoa from one buck, but in a closed stock the minimum number of breeding bucks must be considered in relation to the rate of inbreeding which can be tolerated. This matter will be dealt with more fully in a later section and it will be shown that at least ten breeding bucks should be used each generation. If a strain is to be kept in one hundred cages, then ten are required for breeding bucks and a further five for replacement bucks. Of the remaining eighty-five cages, fifty can be used for breeding does and thirty-five for young stock. Incidentally, it is of considerable advantage to have cages which can be divided or enlarged with movable partitions, as young bucks and does awaiting mating can be housed in much smaller cages than does with litters.

If breeding is carried on throughout the year, an average annual conception

rate of 60% is a very rough guide to what one might expect. The type of stock and standard of management can raise this figure by 15% to 20%, or lower it by 20% or more. Taking this figure of 60% to estimate production; for every fifty does mated, thirty will produce litters. As previously mentioned, the litter size will vary with the strain, but taking a small to medium sized strain with an average litter size of six at birth, the thirty does which have litters will produce 180 young rabbits. Mortality from birth to weaning is in the region of 20%, so that of the 180 animals born, 144 will be weaned. A further reduction of 5% from weaning to 6 months of age can be expected through mortality and culling, leaving a total of 137 sexually mature animals. The average production per breeding doe housed is therefore 2·74 animals per mating. The annual output of rabbits from a colony will depend on the length of the breeding season. The example given above assumes that breeding is carried on throughout the year, and with such a system it is possible to mate the does every 10 weeks so that the expected annual output per doe housed is 13·7 adult animals in a strain having a mature body weight of about 3 kg. The rate at which breeding stock can be built up can also be estimated from this figure. One year after mating fifty does there should be approximately 200 sexually mature does available for breeding.

A ten-week mating cycle can be used even if the litter is not weaned until eight weeks of age, but weaning at such a late stage is liable to have adverse effects on the condition of the doe and her subsequent litters. Weaning at four weeks of age is perfectly satisfactory provided the young are changed to quarters which are warm and draught free. Care must be taken rapidly to reduce the doe's ration immediately after weaning if there is any likelihood of her becoming too fat. A doe which is rather thin when the litter is weaned can be got into good condition in the two weeks prior to re-mating. If difficulty is experienced with re-mating does after two weeks rest it is probably better to delay weaning until five or six weeks and thereafter mate her immediately. The actual programme adopted will be determined by the performance of the strain. At this rate of production the average useful breeding life of a doe is probably two years although a good doe will breed regularly for three years.

Replacement of breeding stock may be on the all-in all-out system, enabling the breeding quarters to be completely emptied and cleaned. Such a system has certain advantages with respects to disease control, but it puts heavy demands on the cage requirement for holding young breeding stock. A more practical system is to run the breeding stock in regular ages. If a total of fifty breeding does can be accommodated, twenty-five six-month-old maiden does can be mated each year. This number would be reduced to fifteen the second year and ten the third year. Using this technique, a certain amount of selection pressure can be applied to improve the breeding performance within the strain.

E. BREEDING PROGRAMME

1. Maintenance of constant genotype

Mating systems to produce inbred lines and thereafter maintain a uniform inbred line have been amply discussed by Falconer (1957).

A strain of rabbits may be used to produce research material or as a control in a selection experiment and in either case the object is to minimize genetic change from generation to generation. The genes which are passed on from one generation to the next are a sample drawn from genes present in the parent population. Genes which are at a low frequency in the parent population may, by chance alone, not be present in any of the gametes which form the subsequent generation. The larger the number of parents contributing to the next generation the greater will be the chance of having all the genes present in one generation represented again in the next. As each sex contributes equally to the next generation, it follows that the maximum number of genes will be conserved in the population when an equal number of male and female parents contribute offspring to the subsequent generation. This subject has been examined in detail by Gowe *et al.* (1959) and the effect of altering the ratio of males to females has been related to the expected change in genetic constitution of each generation. A mating programme in which each male is mated to only one female and each such mating contributes one male and one female offspring to the next generation minimizes change in the genetic constitution by doubling the effective size of the population and thereby halving the rate of inbreeding. The expected change in the mean value of any character controlled by a number of additively acting genes is obtained from the formula $2F\sigma_g^2$ (where F is the inbreeding coefficient and σ_g^2 is the additive genetic variance of the character concerned). It is therefore possible to estimate the amount of change but not the direction for any one or more characters in a strain. Having decided how much change in the mean value of these characters can be tolerated, an appropriate number of male and female parents can be used in the breeding programme.

2. Stock improvement

The question of improving a laboratory strain or maintaining a constant genotype is always extremely difficult to answer and the answer must inevitably depend on the research programme. The maintenance of a control or genetically constant strain is always useful, especially when it is wished to repeat experiments carried out some time previously. At the same time the provision of stock for new experiments must be considered and there is every reason therefore to have a breeding programme for some strains which is aimed at improving either certain characteristics or the overall productivity of the strain.

The efficiency of any selection programme is dependent on a number of different factors, but one of the most important is population size. The

intensity of selection which can be applied in any population is controlled by the rate of inbreeding which can be tolerated. The rate of inbreeding is dependent on the number of parents contributing offspring to the next generation, and having decided that the rate of inbreeding should be not more than say 1·5% per generation, this fixes the number of parents at ten males and fifty females. The selection that can be carried out for any chosen character is therefore proportional to the number of animals from which the required ten males and fifty females can be chosen. If the number of selected parents is altered, the resulting change in the rate of inbreeding can be calculated from the formula

$$\Delta F = \frac{1}{8Nm} + \frac{1}{8Nf}$$

where ΔF = the rate of inbreeding, Nm = the number of male parents and Nf = the number of female parents.

The improvement in any character which one can expect to obtain by selection can also be calculated, and the rate of improvement is given by the formula

$$\Delta G = \bar{\imath} h^2 \sigma_p$$

where ΔG = the rate of the genetic change, $\bar{\imath}$ = the selection intensity in standard units, h^2 = the heritability of the character under selection and σ_p = the phenotypic standard deviation of the character. The rate of progress can therefore be expected to be high if three conditions are fulfilled: a very small proportion of the available animals are selected as parents; the heritability of the character is high; and there is considerable genetic variation in the population. Few characters of economic importance have heritability estimates of over 0·5 while characters closely related to reproductive fitness have heritability estimates nearer to 0·05. In a laboratory population of rabbits where perhaps 200 animals can be accommodated and 100 breeding does is the maximum number that can be housed, the selection intensity cannot be high, especially if the character to be improved can only be measured in one sex; litter size is perhaps the most obvious such character. The heritability of this character is probably in the region of 0·15 (deduced from mouse estimates by Falconer (1955) and pig estimates by Lush and Molln (1942)) $\bar{\imath}$ will be approximately 0·8 if 50% of the tested does are selected as breeding stock and σ_p will be approximately 3·0.

The expected rate of improvement without some form of sib progeny testing of bucks will be

$$\Delta G = 0{\cdot}8 \times 0{\cdot}15 \times 3{\cdot}0$$
$$= 0{\cdot}36$$

One would therefore expect an increase in the mean litter size of 0·36 rabbits per generation. To convert this rate of improvement into improvement per

annum, the generation interval must be taken into account. When the generations overlap, as will occur when rabbits ranging in age from six months to three years are used as breeding does, the generation interval for the population is obtained by calculating the average age of parents when their offspring are born. The annual improvement is then obtained by dividing the rate of improvement per generation by the generation interval. If the generation interval in the above example is 1·5 years then the annual increase in the mean litter size will be $\frac{0{\cdot}36}{1{\cdot}5}$ rabbits per annum.

The various types of selection, and the circumstances in which each should be used, have been fully discussed by Falconer (1957, 1960), who suggests that one of the most useful selection techniques for laboratory animals is within-family selection. The rate of improvement is slow because only half the genetic variation in the population is utilized, but it enables a selection programme to be put into effect in very small populations without incurring a high rate of inbreeding. The individuals selected as breeding stock to produce the next generation are selected on their performance relative to the family mean. Each male parent is replaced by one male offspring and each female parent by one female offspring in the next generation. A certain amount of between-family selection may also be incorporated into this programme, either between dam or sire families, but as already mentioned the effect of between-family selection must be considered in relation to inbreeding.

F. ARTIFICIAL INSEMINATION

The widespread use of artificial insemination (AI) in cattle and pigs has resulted in a considerable amount of research being carried out on all aspects of it. The rabbit is a convenient animal for research on this subject because the techniques for collecting semen samples from the buck and inseminating the doe are simple and reliable.

There is usually no need to train the buck to the use of an artificial vagina, especially if a teaser doe is used. The design of the artificial vagina (AV) has been described by Walton (1945). The required pressure and temperature of the AV may vary slightly from buck to buck and must therefore be determined by trial and error. Generally speaking, the temperature inside the AV liner should be in the region of 40°C (104°F) and although it may be advantageous to lower this temperature by several degrees it should not be raised above 45°C (113°F). It is advisable to use a quiet doe, which is not required for breeding, as the teaser. A stuffed rabbit skin or the AV wrapped in a piece of sack can be used after the buck has become accustomed to the AV, but the use of a teaser doe generally facilitates semen collection from young bucks.

The semen is collected in a small glass specimen tube attached to the AV

liner, and after detaching the collection tube from the AV the semen can be allowed to cool slowly to room temperature before use. The storage time for rabbit semen depends on the initial quality of the sample and it should be used within 24 hours. A technique has not yet been developed for the long-term storage of rabbit spermatozoa by freezing. The volume of the ejaculate and number of spermatozoa per ejaculate varies considerably from one ejaculate to the next and from one buck to another. The quality of the ejaculate, as measured by the proportion of spermatozoa which take up eosin stain and which lack an acrosomal cap, also varies considerably; some of this variation can be attributed to genetic causes. It is therefore possible to estimate the fertilizing capacity of a spermatozoa sample by microscopic examination (Beatty, 1957; Napier, 1960). Depending on the quality of the sample, dilution can be carried out with 0·9% NaCl solution to give an inseminate of approximately ten million viable spermatozoa in 0·5 ml of solution (seminal fluid and saline). The concentration of spermatozoa per ml of ejaculate can range from ten million to over one thousand million per ml with an average of five hundred million per ml in a medium-sized strain while the average ejaculate volume in such a strain might be 0·5 ml.

As previously mentioned, the doe will ovulate approximately ten hours after mating. When AI is used instead of natural mating the doe must be suitably stimulated so that ovulation will take place. It is possible to stimulate the doe with the insemination pipette or by mating her with a vasectomized buck. Neither of these methods is absolutely reliable and it is therefore preferable to ensure that ovulation takes place by giving the doe an intravenous injection of luteinizing hormone. The usual technique is to give 20 to 25 i.u. human chorionic gonadotrophin in about 0·25 ml sterile water. The marginal ear vein is the most convenient for giving this injection and if necessary the hair can be shaved from round the vein and the vein itself rubbed up with a small cotton-wool swab moistened with xylol. If the injection can be made without enlarging the vein, bleeding from the vein puncture will cease almost immediately whereas it may take five or ten minutes to stop the post-injection bleeding if the vein is rubbed up too vigorously.

Insemination pipettes can be made from ordinary glass tubing by first cutting lengths of about 6 in (15 cm), making a slight bend about 2 in (5 cm) from one end, reducing the aperture at this end to approximately 2 mm and finally rounding the other end. A rubber teat is used to suck the inseminate into the pipette and the reduced aperture ensures that no semen is lost.

It may be necessary to have some assistance for injecting and inseminating young maiden does, but generally one person can carry out the whole operation. The doe should be allowed to stand or sit on a small non-slip trolley top, or in a shallow sided tray or box. The actual insemination is carried out by lightly grasping the loose skin close to the tail with one hand and inserting the pipette with the other hand. It will be found that the slight

bend in the pipette makes it easier to negotiate the projecting pelvic bone so that the inseminate can be deposited close to the cervix. The conception rate and litter size obtained after AI is not significantly different from that obtained after natural mating and there is no reason why AI should not be used extensively in the breeding programme. It is, however, advisable to make sure that bucks used for AI will mate naturally if required, since it is not uncommon to find that semen samples can be collected from bucks which will rarely serve a doe. The repeated injection of luteinizing hormone from the same source is liable to result in antibody formation in the doe, so that the hormone injection will not bring about ovulation. If this happens, an alternative source of luteinizing hormone must be used.

G. RECORDING SYSTEMS

There is probably a greater diversity of opinion on recording than on any other subject. Almost every rabbit colony has some form of recording system but no two systems are identical. The object in this section is to suggest some possible systems which might be adapted to meet the requirements of the breeding programme in the colony.

One or other of the systems outlined by Carter (1957) is perfectly satisfactory for most laboratory stocks. Matings and litters may be logged in numerical sequence in one book, or the matings may be entered in one book and the litters in another. The number of each mating may be prefixed with a letter or group of letters to identify the particular strain. Each individual born in a litter is identified by the strain letter, the mating number, a number to indicate the number of the litter within the mating and a letter to identify each animal within the litter; for example *A*/125·3*a* ♀ would identify a female as being born in the third litter from mating number 125 in strain *A*. These systems were developed at the Institute of Animal Genetics in Edinburgh, primarily for mouse recording, but the single book system has been used quite satisfactorily with rabbits. The sex of the animal and its date of birth should be incorporated in any record system, and also a reference number which makes two-way identification of pedigree possible.

With rabbits, it is essential to mark the animal itself so that positive identification is always possible. The most reliable method is undoubtedly to tattoo an identification mark in the rabbit's ear. This system of marking is rather messy and time-consuming, but it is efficient. It may be preferable to use leg-rings or poultry wing-bands with the identification mark engraved on the ring or band, but there is always the chance that the rabbit will lose its identification mark with these systems. The increase in speed with which rings or bands can be applied is probably not worth the risks involved.

Where a selection programme is in operation it is very useful to be able to identify not only the rabbit itself but also its sire and dam without having to refer to records. A complete identification of any animal can be made if a

strain letter and generation number is stamped in one ear and a six-figure code in the other. The first pair of numbers relate to the animal's sire, the second pair to its dam, and the last pair give the number of the individual, e.g., 2*B* 041207 indicates that the rabbit is the seventh born to doe 12 mated to sire 04 in generation 2 of strain *B*. A pedigree record is of course necessary to discover the pedigree of the sire 04 and the dam 12. If animal 07 is a buck which is subsequently used for breeding, it will receive a number according to the number of sires used in that generation, e.g., if sire 2*B* 041207 is mated to dam 2*B* 130416 a mating number is made out, or if this sire is the first to be used in generation 2, and similarly if the dam is the first to be mated to this sire, she is given the mating number 0101 so that the offspring from this mating will be numbered 3*B* 010101, 3*B* 010102, etc. This system can best be used when the generations are distinct (i.e., do not overlap). If the generations do overlap and the breeding stock consists of males and females from several generations, this type of recording system becomes rather complicated.

An alternative to this scheme is to number each animal in chronological order and keep an ear number code book from which the animal's pedigree can be obtained. Apart from having a number stamped in the animal's ear, it is useful to have a cage card for each animal, bearing its identification number, sex and date of birth. In the case of breeding does this card may be used for recording on the cage the date when the doe is due to have a litter and the number born. This information is particularly useful when carrying out routine inspection of does about to have litters.

To establish a mating and weaning routine, some form of visible recording is by far the best; a black-board is all that is necessary. The following table gives a recording scheme which has been found satisfactory for keeping a breeding programme on schedule.

Doe ear number	Mate to ♂ No.	Date mated	Date litter born	No. in litter	Date of weaning	Date for re-mating

It is helpful to use different coloured chalks for expected or future dates and events which have taken place, e.g., the date of the mating may be written in white chalk when the mating has been made and an expected date can be entered for the "Date litter born" in yellow chalk. When the litter is born the actual date and litter size would be entered in white chalk. The weaning date and date for re-mating would be entered in yellow chalk, to be changed to white when these operations were carried out. With this system it can be seen at a glance if a doe has failed to produce a litter or if a weaning or mating has been missed.

Assuming that some book system is used to record matings it is almost essential to have available an up-to-the-minute list of the number of animals alive in each strain and an indication of their utilization. A list written out on a sheet of paper has the disadvantage that as animals die or are disposed of,

the list has to be altered and re-written from time to time. Apart from the work involved in making such lists there is always the chance of making errors every time a new list is compiled. One way is to use a visible strip index system, making out a strip for each animal weaned and removing the strip from the holder when the animal dies or leaves the colony. An alternative is to make out a reference card for each animal weaned. In either of these cases the strips or cards can be assembled in strains or sexes by using markers or colour codes. Similarly the use of the animal can be indicated on the strip or card.

Finally, as an alternative to book recording, all records may be kept on cards. A card is made out when each animal is weaned, matings and litter data are recorded on the individual doe's card, while the does mated to each sire are listed on the sire's card. Family summary cards also can be made out to facilitate selection. If such a card system is used it is certainly worth while using punched cards with holes round the periphery. The animals' own identification number is punched on the card together with its sire number and the number of its dam. Any other records which may be required can be either written or punched on the cards. It is therefore not necessary to file these cards in numerical order. There are of course several disadvantages to punched card recording. Any error in punching data on the card is difficult to trace unless some automatic checking system is employed, and there is always the chance that individual cards will be removed from the file and lost.

H. MANAGEMENT OF BREEDING COLONY

1. Labour requirements

The number of staff required for a rabbit colony cannot be directly related to the number of animals kept, because the layout of the rabbit house, the type of cages and the amount of automation will materially affect the number of technicians needed. Taking as an example a colony of 500 animals of which 100 are breeding does and the remaining 400 consist of breeding males, young stock and mature animals retained for research purposes, two full-time workers are required. This assumes that the stock is kept on solid-floor cages which require cleaning once a fortnight, that food is rationed (and therefore fed daily) and water is supplied by some form of automatic system. A wire cage battery installation with hopper feeding, automatic watering and semi-automatic cleaning may enable the labour requirement to be reduced to one technician for the above colony. However, reducing the labour staff to the absolute minimum is probably false economy in view of our present limited knowledge of rabbit husbandry. It is essential to give considerable individual attention to rabbits, and time spent inspecting the stock is not time wasted. It is surprising how much time is required to keep clean not only the inside of

rabbit cages but also the outside of the cage and the structure of the rabbit house. It is therefore better always to have slightly over the minimum labour requirement.

2. Stock records

It is very easy to become over-zealous with record keeping and one may soon find that a large amount of useless information has accumulated. Having decided what information is essential for the efficient running of the rabbit colony, only the data necessary to give this information should be recorded.

A monthly count of the total stock in each room is useful for estimating cage requirements, and may be related to the monthly food consumption. The stock count can conveniently be kept in conjunction with a running graph on which is plotted monthly the number of animals weaned and the post-weaning mortality. A very useful indication of the health of the colony can be obtained by chalking up on a blackboard an accumulated total of the number of animals weaned and the post-weaning deaths for the month; these totals are transferred to the graph at the end of each month. In a large breeding colony one expects a few deaths each week, particularly in newly weaned stock. It is therefore difficult to be certain when a disease outbreak is just starting unless a close check is kept on mortality.

V. DISEASE

The prevention and cure of disease in the domestic rabbit is a large subject which cannot adequately be covered in this chapter. There are two books which deal with diseases very fully: *The Rabbit in Health and Disease* by J. B. MacDougall (unfortunately out of print) and *Rabbits Ailments* by W. P. Blount (published by *Fur and Feather*) which is still obtainable.

The object in this section is to discuss some of the more important aspects of hygiene, disease prevention and a few of the common diseases likely to be encountered in the rabbit colony.

Rabbits are probably no better and no worse than any other forms of livestock from the point of view of susceptibility to disease. Although much is known about rabbit diseases, their prevention and cure, a relatively small number of well-understood diseases is responsible for a very large percentage of the mortality in the rabbit colony. The course of an outbreak of any of these common diseases can usually be attributed to bad hygiene.

A. HOUSING AND EQUIPMENT

It is obviously essential to exclude all vermin from the rabbit house, as rats and mice are the most likely carriers of disease organisms to invade not only the rabbit house but also the rabbit cages. It is just as important, however, to

make it a rule that no other form of livestock, wild or domestic, should be allowed in. These same observations apply to the food and bedding store if it happens to be separate from the rabbit building. Where hay or straw is being used, mice are very liable to be imported, with baled straw in particular, so it is always advisable to have vermin-proof doors which prevent the entry of vermin into the store and also prevent their getting from the store into any of the rabbit rooms. It is probably worth while having poison bait always present in the food and bedding store, thus ensuring that a colony of unwanted animals does not become established. It is perhaps as well to emphasize that the exclusion of all livestock includes birds; small birds such as sparrows are experts at finding a way into animal rooms where food may be available, and apart from the danger of disease they add greatly to cleaning problems. All ventilators and windows should therefore be suitably screened.

The rabbit house, rabbit cages, and all equipment used in connection with the colony should be kept in a high state of cleanliness and regularly disinfected. Dirt adhering to the surface can be efficiently removed by scrubbing with washing soda solution, or as an alternative a pressure hose or steam weaver can be used. Some disinfectants such as lysol are reasonably effective in the presence of organic matter, while those based on chlorine are inactivated by it. No matter what form of disinfectant is used, its efficiency is reduced unless all organic matter is first removed. Increasing the strength of the disinfectant solution to compensate for this fact is highly undesirable where livestock may come into contact with the solution. A cleaning and disinfecting routine for use in animal houses has been described by Perkins and Short (1957). It is based on the use of Tego, an ampholytic surface active compound —dodecyl-di(amino-ethyl)-glycine-hydrochloride, which can be used either as a spray or in liquid form. The low toxicity of this substance makes it admirably suitable for use in animal houses. It can be used for cleaning everything from the structure of the building to food and water containers. The risk of having an outbreak of disease can be further lessened by ensuring that the stock is always kept in a suitable environment. Apart from being clean and relatively free from pathogenic organisms the cages should be dry, well ventilated (but free from draughts) and, in the case of unweaned rabbits, warm. Young stock are certainly the most susceptible to chilling but adult stock may also suffer in cold damp conditions which, if not corrected, may lead to losses from pneumonia. Animals housed in wire cages are much more susceptible to cold and damp, but even in solid cages it is advisable to provide plenty of clean dry bedding to young stock and breeding does, where the rabbit house is not heated.

Incorrect feeding or the feeding of contaminated food is quite a common source of trouble in rabbits. Greenfoods and hay are liable to carry coccidial oöcysts, flukes, roundworms or tapeworm cysts and for this reason alone it is advisable to avoid feeding greenfood or hay in the ration. Where a properly

balanced pelleted ration is fed there is unlikely to be trouble attributable to nutrition. Little can be done by testing such a ration scientifically to ensure that the pellets are fresh; one must judge by appearance and smell. Pellets which are damp, caked together, or show any signs of moulds should not be fed to any class of stock.

B. COMMON DISEASES

Much can be done to limit or prevent the spread of disease by careful daily inspection of the stock. This can be done at feeding time, which in fact is probably the best time to detect sick animals. Any animal which has not consumed its usual quantity of food or water should immediately be suspect, as should any rabbit which remains hunched up in one corner of the cage. A good animal technician will spot a sick rabbit long before there are any pronounced symptoms of disease, and this is extremely important if that particular animal is to be cured. Rabbits can look quite healthy at night and be dead the next morning.

The incidence of disease in a rabbit colony will vary with the season and the particular diseases to which the animals in the colony are susceptible; similarly there will be seasonal fluctuations in the percentage mortality in the stock.

1. Parasites

External parasites such as fleas, lice and mites should never be present in laboratory stocks of rabbits, but if there is a sporadic outbreak the trouble can rapidly be cured with any of the dusting powders based on gamma benzene hexachloride.

Internal parasites are not usually troublesome in colonies of rabbits which are isolated from other livestock and are fed only a pelleted rabbit ration. Roundworms, tapeworms and cysts are common in domestic rabbits and when importing stock into the laboratory it is quite likely that a significant proportion of purchased stock will be suffering from worm or cyst infection. Roundworms may be cured by dosing with piperazine compounds, but regarding tapeworm cysts, prevention is the only cure.

The internal parasite most likely to cause trouble in the rabbit colony is the protozoan causing coccidiosis. There are two forms of coccidiosis, hepatic, caused by *Eimeria stiedae*, and intestinal, caused by *E. perforans* and *E. magna*.

a. Hepatic coccidiosis. Oöcysts of *E. steidae* which have been excreted by an infected rabbit pass through an incubation period in the bedding or faecal pellets, after which they become infective. If infected oöcysts are ingested by a rabbit, they travel *via* the cells of the small intestine and the blood steam to the bile ducts of the liver. There they multiply both asexually and sexually,

causing the diagnostic lesions on the liver due to dilatation of the bile ducts and thickening of the duct walls. The lesions are quite distinctive as they do not affect the liver cells themselves, being confined to the bile ducts. The lesions may contain a certain amount of bile and will therefore have a green tinge. Microscopic examination of the lesions or bile fluid will reveal large numbers of the coccidial oöcysts. The rabbit may have quite a heavy infestation of coccidia and yet show few outward signs of the disease. The growth rate may be slow and to the experienced eye the animal may look unthrifty.

b. Intestinal coccidiosis. This form may be caused by *E. perforans* or *E. magna* separately or together, and although the life cycle of these two forms is similar to *E. stiedae*, they attack only the intestinal epithelium. The signs are to all outward appearances the same as for the hepatic form; there is no blood in the droppings and in neither case is diarrhoea associated with the disease.

An effective cure can be obtained by treatment with nitrofurazone, chloroquine sulphate, sulphadimidine sodium (Deom and Mortelmans, 1954) or furazolidone (Macdonald, 1957) by adding one of these drugs to the drinking water or having it incorporated in the food. If the disease is endemic in the rabbit colony it may be advisable to have a coccidiostat incorporated in the food at a preventive level. The complete eradication of coccidiosis is a lengthy and troublesome process, because the oöcysts are particularly resistant to most disinfectants. However, it has been shown by Horton-Smith *et al.* (1940) that the oöcysts are susceptible to ammonia and that a 100% kill is obtained in 45 min using a 10% solution of ammonia. The use of wire cages for rearing stock will help in the control of the disease, but it should be realized that animals housed on wire can and do still suffer from it unless all the other points, such as the exclusion of contaminated food, keeping the animals away from their droppings and regular disinfection with ammonia, are strictly adhered to.

2. Bacterial diseases

a. Enteritis. This disease is responsible for a high proportion of the mortality in young stock and so far the cause of the disease cannot be stated with certainty. It has, however, been possible to associate *Escherichia coli* with some forms of mucoid enteritis. When the *E. coli* is typed and tested for antibiotic sensitivity, it may be found that the type varies from one outbreak to another, as well as the sensitivity of the bacterium to antibiotics. This makes it very difficult to suggest a cure as the treatment is specific to the particular outbreak of the disease. Some success has been achieved when bifuran has been given in the drinking water at high levels to cure enteritis and incorporated in the ration at preventive level.

There are also indications that enteritis may be associated with nutrition. Whether the relationship is direct or indirect, as may be the case if a certain ration causes a change in the microbial flora of the intestine, is not known.

Illogical as it may seem, changing the ration during an outbreak of enteritis may markedly decrease the incidence of the disease; e.g. supplementing an all-pelleted ration with greens, or changing from one type of pellet to another. The disease may also be associated with stress factors, as the form of enteritis associated with *E. coli* appears to be more common in the late autumn and winter, mortality being at a minimum in midsummer.

b. Pseudotuberculosis. Tuberculosis proper, as caused by *Mycobacterium tuberculosis*, is rare in rabbits and is unlikely to be of any importance in a laboratory stock. Pseudotuberculosis, on the other hand, is much more common and is often introduced to the rabbit colony by vermin contamination of the food. The causal organism is *Pasteurella pseudotuberculosis rodentium* and there are rarely any marked signs other than a gradual loss in condition followed by sudden death. The lesions are generally widespread through the mesenteric lymph glands and also affect the spleen, liver, sacculus and appendix. Unlike tuberculosis, lung lesions are infrequent.

Prevention is the only really effective way of dealing with this disease, although a certain measure of success may be had by treating affected animals with chloramphenicol.

c. Snuffles. This disease is quite common in rabbits and probably occurs in several forms. Pasteurella organisms are usually associated with snuffles and although affected animals do not normally die, they may become severely affected with pasteurellosis or septic pneumonia which results in sudden death. The diagnostic signs are a nasal discharge consisting mainly of white pus. Some success may be achieved by treating affected animals with a combination of chloramphenicol and streptomycin, but it is difficult to effect a lasting cure. This disease is extremely difficult to eradicate completely, but wherever possible animals showing the least clinical signs should be culled from the breeding stock.

3. Virus diseases

a. Myxomatosis. There are few known diseases in the rabbit attributable to virus infection, and the only one of importance is myxomatosis. This disease is spread either by direct contact or by biting insects and mites and should therefore never be a problem in a laboratory rabbit colony. If the disease does break out, its spread is likely to be slow, and immediate action will prevent heavy losses.

The incubation period is approximately one week and the signs of the disease are unmistakable. First there is a swelling above the eyes and a mucoid sticky discharge from the eyes. Soon after the appearance of these signs, swellings appear on various parts of the body but most diagnostically on the genitalia, at the base of the ears and round the eyes and nose.

The disease cannot be cured, so all infected stock should immediately be

killed. Vaccination against myxomatosis is possible but probably not worth while. Rabbits have been kept in a house from which insects could not be excluded during two outbreaks of the disease among the local wild population. Rabbits suffering from myxomatosis were found within yards of the house, but not one rabbit inside contracted the disease. Insects were kept at a minimum by regular spraying with insecticides, while the cages and litter were treated with gamma benzene hexachloride dust.

4. Nutritional disorders

Little is known about the cause of the various nutritional disorders from which rabbits are liable to suffer from time to time. Even when there is no known change in the ration, losses may occur and the animals show no signs other than an enteritis and perhaps diarrhoea. Where no pathogenic organism can be isolated, it must be assumed that the diet is at fault. Such disorders may be cured by changing the ration for a short time. This hit-or-miss cure has been known to work not only with rabbits but also with poultry, where a great deal more is known about nutritional requirements. It is difficult to suggest why one pelleted ration should cure the trouble caused by another. A likely reason is that some of the ingredients in the ration have been changed, giving a new ration which on crude chemical analysis is the same but is biologically different.

The feeding of green food, especially lucerne and clover, may cause bloat in rabbits. The proportion of greenfood in the ration should immediately be reduced and if possible a pelleted ration alone should be fed for a short time, thereafter gradually reintroducing greenfood. Little can be done to cure affected animals as the progress of the disorder is usually very rapid, ending in death. Bloat can occur at any time of the year, but is most common in summer when very lush green food may be fed.

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Chapter 12

Hamsters

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I. INTRODUCTION

The Syrian (golden) hamster, *Mesocricetus auratus*, is the most recently introduced laboratory animal to become widely used for research. It has, in addition, become popular in the non-professional community, with both adults and children alike, thousands being sold each year as household pets. Despite evidence that the golden hamster can be gentle and engaging, it has been known for technicians with bleeding fingers and frayed tempers to complain bitterly about the animal's disposition. Little by little, information has accumulated about this creature of ambiguous nature.

A. HISTORY

The Syrian hamster was originally described by Waterhouse in 1839 as a deep golden yellow in colour, with fur moderately long and very soft. He stated that the fur was a deep grey or lead colour at the base on the dorsal surface of the animal and faintly grey at the base on the ventral surface. The ears had deep golden-coloured hairs on their outer surface, and white hairs on their inner surface. The adult males are larger than the adult females and possess a well-developed hip gland which is a darkly pigmented spot in the hip region with roughened skin and coarse, darkly coloured hairs. It is apparently glandular and especially active in the male. When he becomes sexually excited the fur above the gland becomes wet, and he scratches and

rubs this spot as though the secretions were irritating to the skin. In the female the gland is poorly developed and scarcely visible, yet there is some evidence from her behaviour that it secretes during the time she is in heat. The skin of the hamster is loose and abundant and hides the finer contours of the body.

After the original introduction of the Syrian hamster to the London Zoological Society in 1839 by Waterhouse, it came next to the attention of the scientific world when Professor Aharoni of the Department of Zoology of the Hebrew University, Jerusalem, captured a litter of eight young animals which he reared and presented to the Department of Pathology of that University in July 1930. Of these eight litter-mates, four escaped, one female was killed by the male and but three animals (one male and two females) survived to breed (Adler, 1948). These animals and their descendants were used for investigations on kala-azar and proved so successful that as soon as Dr. Adler realized their value for laboratory use he decided to distribute them as widely as possible. He took specimens to the Collège de France, to the Medical Research Council (U.K.) and to the Wellcome Bureau of Scientific Research, London, where they were successfully bred by Dr. Hindle. Other animals were later sent to India, and still others to America and Egypt. Dr. Adler reported:

> To the best of my knowledge all the golden hamsters now in use as laboratory animals in Europe and America originate from the above-mentioned three litter-mates—one male and two females brought to the Department of Parasitology of the Hebrew University of Jerusalem in July 1930.

In the excellent hamster bibliography compiled by Magalhaes (1959b) covering the years up to 1959, one can find 14 references to hamsters during the 100 years between 1835 and 1935. For the next ten years (1935-1945), 143 papers are listed; and in the ten-year period between 1945 and 1955, the number of reported studies on Syrian hamsters is 1,070. It would be difficult to estimate the number of publications appearing since 1955 whose direct subject has been the Syrian hamster, or whose aurstho have used this species as their research animal.

B. RELATED SPECIES

The two species of hamsters most frequently referred to in connection with the Syrian hamster, *Mesocricetus auratus auratus*, are the European or black hamster, *Cricetus cricetus* (*C. vulgaris*, *C. frumentarius*), and the Chinese or grey hamster, known variously as *Cricetulus griseus*, *Cricetulus barabensis griseus* or *Cricetus griseus* (Hindle and Magalhaes, 1957). According to Granados (1951), the term Syrian hamster may be misleading since there are two varieties found in the same area, the second one being *Mesocricetus auratus brandti* Nehring. According to Wahrman (1959), this variant has two fewer chromosomes than *Mesocricetus auratus auratus*.

Cricetulus griseus and *Cricetus cricetus* both possess a diploid number of 22 chromosomes whereas *Mesocricetus auratus* has twice as many, or 44 (Sachs, 1952; Gates, 1953). Despite the similarity in the number of chromosomes for both *Cricetulus griseus* and *Cricetus cricetus*, these two species differ considerably from one another in size, colour and distribution. The Chinese hamster ranges from the eastern coast of China to the eastern shore of the Caspian Sea. The European hamster is found from the western coast of Europe to a point roughly north of India in its extreme eastern extent. Where these two ranges overlap, west of the Caspian Sea, and in an area lying between the Caspian and Black seas, the Syrian hamster, *Mesocricetus auratus*, is found. Its distribution is much more limited than that of the Chinese and European forms, and its appearance as a distinct, new species, occurred much later in geological history. Because of these and other facts, Sachs (1952) concludes that *Mesocricetus auratus* is a tetraploid arising from hybrid crosses between *Cricetulus griseus* and *Cricetus cricetus*.

In size, *Mesocricetus auratus* is midway between *Cricetus cricetus* which is considerably larger, being about nine inches (23 cm) long (Eaton, 1948) and *Cricetulus griseus* which is four to five inches (10-13 cm) long. The golden hamster is about six inches (15 cm) long when adult. The European or black hamster is light brown above, black below and white at the sides. The Chinese hamster is grey above with a black stripe down the centre of its back, and light below. The Syrian hamster is light below, golden brown above and has very little black except where it borders the white cheek-flashes. The black and grey hamsters have 8 mammae, but the tetraploid golden hamster has from 14 to 22 mammae (Hindle and Magalhaes, 1957; Sachs, 1952).

The Chinese hamster was first introduced to laboratories in 1919 by Hsieh (Yerganian, 1959), where it was used in the study of kala-azar. This species is particularly useful for cytogenetic studies by virtue of its haploid chromosome number of 11, which greatly facilitates the study of linkage groups. The chromosome types are easily recognized (Husted, Hopkins and Moure, 1945) and the X- and Y-chromosomes are larger than the autosomes, containing much heterochromatin which can be seen in mitosis. The animals are easy to care for, odourless, docile to handle, and will breed throughout the year if managed properly.

The areas of research in which the Chinese hamster has been used include experimental infectious studies on leprosy and ricksettsia, irradiation-induced chromosome aberrations, cheek pouch vascular studies (the pouch being thinner and more transparent than that of the golden hamster), sex chromosome cytology and others (Yerganian, 1959). A recent apparent mutation has led to the establishment of a line of diabetic animals.

Ritterson and Mauer (1957) have demonstrated that the normal Chinese hamster, unlike the golden hamster (Knigge, 1954), has adrenals which have uniformly distributed lipid materials in the cortex, and responds to chronic

stress more like other laboratory animals in its adrenal response than lik the golden hamster to which it is more closely related. Parkes (1931) published an account of the reproductive cycle in the female Chinese hamster, reporting that the length of the oestrous cycle is four and one-half days, with oestrus lasting one and one-half days.

C. USES IN THE LABORATORY

The first use of Syrian hamsters for research was in 1930 when they served as successful experimental hosts for the organisms causing kala-azar. In fact, it was through their use that 20 years of fruitless search for the mode of transmission of this disease came to a successful conclusion (Chandler, 1949). Since that time they have continued to be widely used for parasitological and bacteriological studies and have in addition become useful as experimental hosts for viruses such as those of poliomyelitis, Newcastle disease, canine distemper, mumps, blue tongue of sheep, equine encephalitis, Colorado tick fever, herpes simplex, equine abortion, influenza, rabies and others (Magalhaes, 1959b). Because of its ability to develop dental caries under proper conditions of diet and oral flora, the hamster is much used in dental research (Keyes, 1949). Since hamsters hibernate in response to cold weather, they are well suited to studies of natural and induced hypothermia and its effects upon mammalian physiology (Lyman, 1950, 1955). The presence in these animals of a naked (non-furry) cheek pouch has given scientists a living laboratory for the study of microcirculation (Fulton and Lutz, 1957). The reproductive processes of the hamster have had particular attention because of its unusually short gestation period of 16 days as well as the remarkably precise timing of the female cycle (Orsini, 1961). The adrenal of the hamster differs from that of other laboratory animals in the histochemical responses of its stainable lipids during periods of stress (Knigge, 1954). As mentioned above, it, as well as the Chinese hamster, has been used for the study of mammalian chromosome complexes (Husted *et al.*, 1945; Yerganian, 1953). The golden hamster has been used by Billingham and his co-workers as a subject for immunogenetic studies (Billingham and Hildemann, 1958). It is interesting and a bit puzzling to note that reports of classical genetic studies have been slow in appearing, considering the length of time this animal has been common in laboratories and pet shops. Recently, because of its unusual immunogenetic responses, the hamster has become useful for the screening programmes of those laboratories hoping to discover effective chemotherapeutic treatments for malignant human tumours.

D. ANATOMY AND PHYSIOLOGY

The uses briefly outlined above to which the hamster has been put have depended upon its anatomical peculiarities and physiological responses, many of which are remarkable if not unique among laboratory animals.

1. Anatomy

a. Cheek pouch. There are many rodents that possess cheek pouches, but the size of the pouch together with the absence of fur on its surface is characteristic only of the true hamsters of Europe and Asia (*Cricetulus*, *Phodopus*, *Cricetus*, *Mesocricetus*) (Priddy and Brodie, 1948).

Priddy and Brodie (1948) have made a detailed study of the innervation, vascularization and musculature of the pouch. They describe the pouch as an evagination of the lateral buccal wall which is devoid of glands and possesses no vascular papillae except at the aperture. The walls of the pouch are provided with longitudinal musculature which consists mainly of extended portions of *musculus buccinatorius*. The action of the longitudinal musculature is to contract the pouch and thus draw it towards its aperture, presumably as a means of helping to empty the pouch of its contents. There is also a retractor muscle which prevents the accidental eversion of the pouch and helps to support the pouch when full of stored materials.

The pouch is highly distensible, having longitudinal folds which make this distention possible. When relaxed and empty, the pouch is about 1 cm wide; and from 3 to 5 cm long, depending on its degree of distention with food or other materials.

Its histology has been described (Fulton, Jackson and Lutz, 1947) as having four layers: stratified squamous epithelium of two to five layers of cells, rather dense fibrous connective tissue, longitudinal striated muscle fibres (absent from the blind end of the pouch) and loose areolar connective tissue. Blood vessels are numerous in both muscle and connective tissue layers. The thickness of the fixed and stained pouch was found to be from 0·4 to 0·5 mm.

By using blunt forceps to avoid injuring the membrane, this pouch can be pulled from the animal's mouth while the hamster is under anaesthesia, and stretched over a transillumined stage for observation, thus forming a living laboratory for study of tissues (Fulton and Lutz, 1957). While in this everted position, the pouch can be used as a site for the implantation of autologous, homologous or heterologous tissues. Many studies on tumour growth have been made by using the pouch as an implantation site. Its advantage in such studies is the ease with which the pouches can be everted at desired intervals to measure the growth rate of the implant (the hamster being under anaesthesia). At the same time one can observe the response of the host's tissues and blood vessels to the presence of the malignant tumour. In all such studies, if one pouch is used as an experimental site, the pouch in the opposite cheek can be left intact as a control.

There has been some discussion concerning the presence or absence in the pouch of the same amount and kind of lymphatic drainage present in other tissues of the hamster's body. It is true that this organ is poor in such vessels, it may therefore be an immunologically privileged site, and its use would greatly modify the conclusions one might draw from immunogenetic studies

utilizing homologous or heterologous transplants to this site (Hildemann and Walford, 1960).

b. Viscera. Jung (1958) gave a brief summary of the internal anatomy of the hamster, pointing out the peculiar structure of the stomach. A distinct constriction divides this organ into two portions, the forestomach (cardiac end) and the glandular stomach (pyloric end), with a short, narrow passageway between. The oesophagus enters the cardiac end just ahead of the dividing stricture and so close, anatomically, to the pylorus that the smaller curvature of the stomach is almost non-existent.

Walls (1942) described a special conducting tissue of the heart of the hamster and pointed out the large Purkinje fibres in the sinu-atrial node which was itself of considerable size. During the course of his investigation he recorded the heart rate of a hamster under anaesthesia as 450 beats per minute, using the electrocardiograph.

The orbital sinus of the hamster has been found by Pansky, Jacobs, House and Tassoni (1961) to be as convenient a source for blood from this species as it is in the rat. This sinus is a venous pool which is formed by the junction of the ophthalmic veins and can be reached by retracting the upper lid of the eye and inserting the tip of a hypodermic needle against the bulb capsule, midway along the superior border of, but underneath, the upper lid. The point of the needle is directed downwards and posteriorly as well as inward (medially) toward the back of the orbit. A 4-mm penetration will bring the needle point to a halt against a shelf of bone, and at this location the point will now be within the venous pool provided the needle is withdrawn very slightly. Using this technique to obtain blood samples, House, Pansky and Jacobs (1961) studied the blood values of hamsters ranging in age from four weeks to one year. They found that the fibrinogen of hamster plasma is linked to gamma globulin. When the two are separated, it appears that the normal hamster is low in gamma globulin. They suggested that a decrease in the albumin/globulin ratio which occurs with increasing age may be related to kidney and liver pathology.

The values in Table I were obtained from autopsies performed on animals of three inbred lines and will give an indication of the organ-weights one may expect to find in adult hamsters of similar genetic makeup (Whitney, 1961).

2. Physiology

a. Nutrition. A number of studies has been made on certain nutritional requirements of the hamster in the laboratory and Spector (1956) lists most requirements with the exception of minerals. Although it is known that Syrian hamsters are omnivorous in nature, no studies, to our knowledge, have been done recently to determine what foods they take in their natural habitat. The report which most closely approximates such a study is one appearing in 1945

TABLE I

Organ weights relative to body weights in inbred Syrian hamsters 182 days of age. (Values for organs are as milligrams per 100 grams of body weight)

Designation of inbred lines:	X.68	1·26	5·1
Average body weight in grams:	124·6	117·0	112·7
Number of animals:	(12)	(15)	(19)
♂ gonads	2821·0	1905·0	2309·0
♀ gonads	34·5	29·8	22·1
Pituitary	3·6	3·6	3·9
Spleen	157·9	115·2	113·7
Adrenal	16·8	17·1	19·5
Kidney	863·0	1045·0	817·5
Liver	4574·0	4625·0	4580·0
Thymus	22·4	25·3	19·5
Heart	447·0	418·0	353·5
Lung	556·0	644·0	614·0
Thyroid	6·0	5·6	8·2
Hibernating gland	249·8	168·0	209·0
Eyeball	80·5	77·4	82·7

by Jacobs. One of his hamsters escaped in his apartment, and when it was captured, it was found to have in its cheek pouches, "13 sow bugs, 7 ants, 4 cockroaches, 2 flies and 1 hornet". Jacobs then conducted a small experiment and by it demonstrated that hamsters will choose insects voluntarily, even when other foods are present. An excellent monograph on experimental studies in nutrition and reproduction of the hamster was published by Granados in 1951 and contains an extensive bibliography. He observed that there were seasonal variations in the normal growth curves of these animals, and that the curves differed somewhat in character, moreover, between males and females. Seasonal changes in climate were also found to affect fertility. Regarding the requirements of hamsters for specific dietary elements, he reported others as finding vitamin C necessary in the diet and he himself found that the hamster does not require dietary inositol, biotin, p-aminobenzoic acid, nicotinic acid, folic acid or vitamin K for growth. They did grow faster, however, if given choline in their food. Vitamin B_{12} was found not to be necessary in the hamster's diet, and Granados concluded that the intestinal flora are therefore undoubtedly responsible for synthesizing sufficient amounts of this vitamin for the animal's needs.

Houchin (1942) and West and Mason (1958) have reported on vitamin E and its relation to muscle degeneration in the hamster, and several authors, including Granados (1951) have observed a great susceptibility of the hamster to vitamin E deficiency. The symptoms are, in addition to severe muscular degeneration, loss of weight and early death.

From the scarcity of reports in the literature, it would appear that there is still much to be learned about mineral requirements in this species.

It is well known that hamsters can be maintained adequately in large colonies by feeding standard laboratory chows, but better reproductive

performance and health can be obtained if dietary supplements are given. Many colony operators claim that these animals must have some kind of fresh greens or fruit daily; and others that young, pre-weanling animals especially must be given moist mash or fresh moist vegetables or fruit to prevent an early diet of dry pellets (together with insufficient fluids) from bringing on a stoppage of the bowels and death. Paradoxically, the constipation brought about by too much dry food and too little fluid intake may sometimes result in what appears to be diarrhoea—a wetness about the anal region. The reason for this is not known. (See comments also under *Disorders*, p. 382.)

b. Response to drugs. Granados (1951) lists a number of workers who have contributed to the information we have on hamsters' responses to drugs. Among those mentioned is Chen *et al.* (1945), who tested a variety of anaesthetics and toxins in hamsters. He found that the hamster can be effectively anaesthetized with the common barbituric acid derivatives, for which he stated doses which can be used as guides. He cautioned, however, that the end effects may differ from laboratory to laboratory due to differences in age of experimental animals, environment, handling, etc. In our own laboratory we have found the following doses of veterinary sodium nembutal (Abbott) (phenobarbitone sodium) useful for surgical anaesthesia. The nembutal is made up in a solution containing 60 mg of the drug per ml (Table II). These

TABLE II

Doses of veterinary sodium nembutal (phenobarbitone sodium) suitable for surgical anaesthesia in Syrian hamsters*

Weight of animal in grams	Dose of sodium nembutal in ml
40–50	0·07
51–57	0·08
58–65	0·09
66–69	0·10
70–74	0·11
75–78	0·12
79–85	0·13
86–97	0·14
98–106	0·15
107–115	0·16
116–130	0·17
131–150	0·18
To sacrifice:	0·50
To counteract an overdose:	0·20 picrotoxin

* 60 mg sodium nembutal per ml solution.

doses, administered intraperitoneally, will induce deep anaesthesia in about five minutes, and will maintain the hamster in this condition for about 20 minutes. Booster shots of about 0·02 ml may be given to continue the deep anaesthesia, but should be used with caution. A little experimenting may be

necessary to determine the most effective booster dose, as well as the most effective anaesthetic dose.

c. Hibernation. In one respect the hamster differs physiologically from all other common laboratory animals. It hibernates in response to environmental temperatures of about 48°F (9°C) or below. Lyman and his co-workers have made accurate and detailed studies of the physiological state of hibernating hamsters and have recorded many bodily changes that are unheard of in non-hibernating animals under normal or induced hypothermic conditions.

The heart rate of hamsters in hibernation may be as low as six beats per minute (normal rate, 200 to 300 per minute), and respiration consists of periods of apnoea lasting several minutes, followed by several deep breaths. The rate can vary from 0·46 to 0·9 breaths per minute (Lyman, 1951).

It should be emphasized here that because low environmental temperatures have such a profound effect on hamster physiological processes, it is important that any room where they are held for breeding or experiment, has thermostatically controlled heat, and insulation, to maintain the temperature at a reasonably constant level.

E. REPRODUCTION, EMBRYOLOGY AND GROWTH

1. Reproductive cycles

Many have studied the oestrous cycle of the female hamster including Deanesly, 1938; Kent and Smith, 1945; and Ward, 1946, 1948. Orsini (1961) has amply demonstrated the presence of an exact 4-day cycle which follows such a constant pattern in individual animals that once this pattern is established one can plan research for months ahead, knowing the onset of oestrus can be predicted precisely. The technique involved could be of great advantage when it is necessary to arrange for dated matings so that new-born young are available on a specific date for experimental treatment, or for the study of accurately identified embryonic stages.

Using only the finger-contact technique of judging vaginal discharge, one may rapidly and accurately evaluate the stage of the cycle, and the technique can be learned easily by technicians and handlers in commercial or experimental colonies.

Briefly the cyclic changes, as described by Orsini (1961) for animals reared by normal daylight, are as follows. On the morning of day 1 there is a slight, translucent mucous discharge which increases in amount as evening approaches. It builds up in opacity and quantity from evening of day 1 to morning of day 2, and it is during this interval that oestrus occurs. By the morning of day 2 the postoestrous discharge may protrude from the vaginal orifice or may flow from a sealed vaginal orifice upon applying slight pressure as the animal is picked up for observation. It is usually copious in amount, very viscous (adhering to the finger and stringing out) and has a distinct

odour. This discharge occurs regularly every fourth day on the morning following oestrus. By afternoon of day 2 it has lost its viscous (stringy) characteristic and resembles a white, waxy plug. On day 3 this waxy plug when present is distinct, has an irregular outline and is yellowish in colour, though it may be lost from the vagina some time during the day. If this plug is lost on day 3, the vaginal discharge on day 4 may be moist and non-mucous; but the plug may still be present. By the morning of the following day (now day 1 again) the discharge has changed from a non-mucous one to the distinctly mucous or viscous fluid typical of the pre-oestrous discharge. It is fine, light, like a cobweb, and strings out for only a short distance after contact by finger. Copulation can occur at any time from the evening of day 1 to the morning of day 2.

Ovulation occurs in hamsters between one and three in the morning of day 2 (Ward, 1948; Orsini, 1954; Strauss, 1956). Chang and Shaeffer (1957) reported that the male hamster will copulate, when the female is in heat, about 50 times within an hour. They found that sperm cells are ejaculated in most of these copulations and that the first five copulations produced fewer sperm, the eleventh to thirtieth copulations producing the greatest number. Yamanaka and Soderwall (1960) have carefully timed the arrival of sperm in the various levels of the female tract by performing autopsies at two-minute intervals immediately following copulation. They reported that the shortest time after mating at which spermatozoa were observed in the ampulla of the oviduct was 91 seconds. Krehbiel (1952) found that female hamsters will accept the male while pregnant, and that the time of this acceptance corresponded to the expected oestrus period had there been no pregnancy. However, in many of these animals, no sperm or ova were found in the tubes after copulation and no young were found which could be attributed to this mating. None of the females he studied mated during the period immediately following birth of their young or during the lactation period.

There are many factors which may influence the number and size of litters produced by individual females. A large littering cage, 12×24 in (30×60 cm), is said to encourage the production of large litters. Poiley (1960) has shown that seasonal variation can affect litters as well as the order of the litter in the sequence of litters produced by the mother. The average size of a female's first litter, he found, was 9·81+, of her third litter was 10·33− and of her seventh litter was 4·58+. Females will raise a litter of one (rarely) or a litter of 16 or more (also rarely), and the factors which determine whether she will raise all or only part of the number born are obscure. In our own experience, if a litter is small in number (four or less) the mother is very likely to kill all the young. Whether this is indicative of poor physical condition on the part of the mother or lack of stimulation by the nursing young is not known. There may be still other factors at work as well. The system of breeding will greatly influence the productivity of a colony. Outbreeding, as is well

known, will result in litters larger in size and with more vigour than will inbreeding. An inbred colony may average but six young weaned per litter (Whitney, 1961), and yet one hears reports of an average litter size of 16 in colonies outbred and selected for litter size. It is reasonable to suppose that females selected for a greater number of mammae would be more successful in raising large litters.

Whether the diet of the mother is adequate may have a great influence on the size of the litter weaned. It is important that the young have access to fluid other than the mother's milk, and for this reason it is vital that the spout of the water bottle be low enough for young hamsters to reach it when they are old enough to wander from the nest. Females will generally kill their young if allowed to bear them in a cage with other adults and disturbing the mother at the time of parturition will have equally disastrous results. On the other hand, daily handling of female hamsters may gentle them to the extent that they may be picked up during or shortly after the birth of their young and still care for them normally (Orsini, 1961).

It is theoretically possible for a female hamster to have 7 or 8 litters per year; the more usual number is 5 to 6. Where stock is being inbred for experimental use, one can expect about 4 generations a year. Hence, to raise an inbred line of 20 generations may require a minimum of five years. In actual practice it may take longer, for in our own experience we have found that the higher the degree of inbreeding, the greater the difficulty we have had in obtaining prompt matings as the young females and males mature. This difficulty is apparently due to the so-called sterility factors which increase in their frequency of expression as inbreeding progresses. What these factors may be is not known. In some lines, at least, the resulting sterility appears more frequently among males than among females. We saved one inbred line by breeding young females back to their sires, for their sibling males frequently produced no young by their sisters, and were later found to be unproductive with non-sibling females as well.

Advanced age on the part of the mother has an effect upon litter size as was shown by Soderwall, Kent, Turbyfill and Britenbaker in 1960. They found that from mothers up to the age of 13 months, litters did not vary significantly from one another in size. Beginning with 14 months of age, however, females produced litters whose sizes were reduced markedly. At 13 months the mean litter size was 6·9; at 14 months the mean litter size was 3·8; and the average mean litter size from 15 through 27 months was 1·02. In addition to the decrease in the number of young born per litter as the mother grew older, it was also found that the length of the gestation periods became significantly greater. From one through 13 months the length of gestation in hours ranged from 373 to 393; beginning with the 14th month the gestations ranged from 397 to 443 hours in length. One might suspect, they say, that the small litters were due to a reduction in ova produced and released by the

ovaries. However, histological sections showed that ovaries contained the normal number of follicles and uteri contained the expected number of implantation sites. The explanation for reduced litter size therefore rested on the suggestion that the uterine tissues or placental nutrition was insufficient for sustaining the foetuses to term. This theory was supported by the finding that the weight increases of the older females were constant until two or three days before parturition, when their weights dropped suddenly, indicating possible resorption of foetuses.

There is some difference of opinion concerning the age at which hamsters are mature enough to breed. Females will mate at the age of eight weeks or even earlier in some instances. Selle (1945), for example, reported the unusual finding of a female hamster sexually mature at 28 days of age. This, however, is rather rare. Males will be capable of begetting young at the age of eight weeks; however, some breeders prefer to wait another month before placing young males and females together (Cook, 1949; Hindle, 1947). Once a female has weaned her litter, it is a matter of opinion, again, whether she is to be mated immediately or whether she will be allowed to rest for a week or 10 days before re-mating. Some say that a rest period between litters tends to result in fat accumulation which coincides with a decrease in the female's breeding capacity. Others declare that better animals are produced from mothers which have had adequate rest between litters. The choice often rests upon such considerations as whether the females are being bred for commercial purposes, or for pet and show stock.

Studies by Ortiz (1955) have shown that the female hamster enters senility between one and two years of age. She found, however, that the ability of the ovary to respond to the stimulation of injected gonadotrophin (PMS) was very little reduced in senescent females. Hence, though sterility may begin at any age between 11 and 19 months, ovarian involution is not complete at this time. Spagnoli and Charipper (1955) found that aging brought about dedifferentiation and degeneration of germ cells in the tubules of the male testis as well as replacement of some of the tubules by connective tissue. During the course of their histological study they discovered spermatozoa in the testes of young animals above one month of age, as well as motile sperm from smears.

2. Embryology and growth

To produce a hamster from a one-celled ovum takes 16 days—only a bit over two weeks.

The egg cells of hamsters, which are more rich in yolk than are the ova of other mammals, make their way quickly through the oviducts so that on the third day *post coitum* they can be found in the uterine horns (Jung, 1958). Samuel and Hamilton (1942) found that the ova were in the four- to eight-cell stage as they entered the uterus. Orsini (1954), using timed matings, found

more precisely that ova enter the uterus at approximately two days and nineteen hours of developmental age, with implantation beginning at four days eight hours (Orsini, 1948). Implantation was completed by five days six hours. Boyer (1948), commenting on this seeming delay in the initiation of cell division, points out that "once embryogenesis gets under way it proceeds very rapidly for about 36 hours . . . as though trying to recapture the time lost by the delay in implantation".

Orsini's studies (1954) showed that at day 9 circulation begins and the limb buds are present. At about nine and one-half days development slows somewhat and the embryo is spirally coiled at this time (Boyer, 1948). At twelve days the hair follicles begin to appear, and at day 13 the claws have erupted and the eyes are nearly closed. On day 16, just before birth, the skin is tough, loose and wrinkled. There is a profuse covering of hair follicles over the skin, and where the vibrissae patches are located, short bristles have erupted. These, however, are the only hairs present; no others are to be found on the surface of the body despite the roughening of the skin by the presence of the large follicles.

Various phases of embryological development in hamsters have been made the subject of special studies. Purdy and Hillemann (1950a, b, c) for example, reported a series of observations on prenatal growth, mortality and volume changes in the amniotic fluid. They found that a measurable increase in weight of the foetal hamster begins at day 9 and continues to term, with averages ranging from 0·0024 to 2·052 g. They found also that crown-rump measurements increased coincidentally with weight increases and varied from 4·5 mm to 21·32 mm. The mortality of hamsters during the pre-natal period ranged from zero to 77% with the lower mortality being characteristic of the earlier stages of embryonic growth and the higher percentages of death occurring in increasing numbers as parturition approached. These later deaths were most numerous from day 12 to day 16. The litter size of viable embryos most frequently encountered was 10 in number of young, and appeared in 20% of the 72 pregnancies which were studied. The production of amniotic fluid coincided with increase in weight and crown-rump measurements on day 9. Surprisingly, on day 16, at term, the fluid dropped to 53% of the maximum value attained earlier, and became viscous in character.

Boyer (1948) studied the development of the circulatory system in hamsters. By following the embryological stages, he was able to determine that the major vessels in this species were nearly identical to those of the rat.

Other embryological studies have included a study of ossification of the middle ear by Van Ardell (1951); the extraembryonic cavities (Foote *et al.*, 1954); the formation of the pharynx and ultimo-branchial body (Klapper, 1950); development of the pituitary (Krol, 1949); the vitelline and allantoic placentas (Adams and Hillemann, 1960); and the wolffian and mullerian ducts (Ortiz, 1945). Ramm and Swartz (1955) found that the hamster is

unique with regard to its metanephros, for the entire period of its development from anlage to completed units was 4·5 days, "the most rapid of any mammal yet investigated". Lindh (1961) conducted experimental and morphological studies by which he was able to determine the quantitative aspects of pre-natal growth of the gonads in both rats and Syrian hamsters. This report contains a wealth of tabulated data, and an extensive list of references relative to this field.

When young hamsters are born they weight a little more than 2 g; at five days they weigh about 5 g, at 10 days about 10 g, at 15 days about 20 g and at 21 days, when they are usually weaned, the average weight is 34 g (Rosenberg, 1948).

According to a study done by Marx in 1949 on a statistical analysis of the growth of golden hamsters, the mean weight of males at 20 days after birth was 35·84 g, of females, 35·70 g. When nearing maturity, at the fifty-fifth day after birth, however, the weight relationships shifted. The males were then found to average 99·89 g and the females 103·23 g; and from this point on, the females weighed more than the males. On the seventieth day after birth, for example, the mean weight of 64 females was 117·94 g+14·46 and for 71 males of the same age, the mean weight was 108·23 g+13·08.

II. Breeding Methods

There seem to be as many breeding methods suggested or practised with golden hamsters as there are people who oversee their breeding. There are, however, two major methods which are in use, subject to individual variations.

A. test breeding

This method, also known as hand breeding, requires that females be placed with males when the females are thought to be in heat, the pair then being observed until mating occurs or until it is obvious that the female is not receptive or begins to harass the male. If mating does occur, copulation may continue from five minutes to an hour. Generally, by the end of 30 minutes of active mating the male tires. The female, however, is still receptive and she may tease him to the point of physical injury if he is not removed. For this reason it seems best to separate males from females after a half-hour of mating activity, for this length of time appears to be sufficient to ensure a successful impregnation, but not long enough to allow the female to intimidate the male if she becomes aggressive towards him. The advantages of such a breeding method are obvious. It produces litters which are accurately timed, whose day of birth can be predicted and whose sire is known. It prevents the loss of good breeding males through injury by aggressive females, and since a smaller

battery of males is required than for continuous pen breeding, there is a great saving in cages and space.

Always remember one rule: the female is placed in the male's cage for breeding.

B. PEN BREEDING

Some breeders feel it is advantageous to put females with males overnight, removing the females to their individual cages the next morning. This does away with the necessity of staying with the animals to observe whether mating has occurred, and it assures the identity of the sire if a litter is produced. The females, however, may or may not have been in heat (unless tested by vaginal smear or identification of vaginal discharge). If the females are not known to be in oestrus, allowance must be made for this in planning the number of females to be placed with males, provided the breeder requires a minimum number of litters from this date's breedings. Furthermore, the breeder has a 10- to 12-day wait before he can be sure that the females are pregnant, and in the meantime those which are not are idle. The litters obtained from such matings are timed with sufficient accuracy for most laboratory uses, but there is always the chance that the female, during the hours she has been with the male, has done him some injury. Males severely harassed by females may become so timid that they are spoiled for further breeding purposes.

Other breeders use a method whereby the female is caged with the male for a four-day period, assuming that within this period she will have come into heat at least once, and a successful mating has thereby been assured. The advantages are knowledge of the sire, and approximate time of mating within a four-day leeway. These advantages are scarcely sufficient to offset the disadvantages which apply to all methods of pen breeding.

Although one may pen breed pairing one male to one female (monogamous), one male to more than one female (polygamous) or several males and females together (communal), it is not to be recommended because of the frequency of fighting which may occur, with damage not only to males but also to females where more than one is placed in a cage.

There is only one way we know of to reduce the amount of fighting which occurs when several adults are penned together. If the animals which are to be caged in one pen are raised together in that pen from the time of weaning, the fact that they all possess the same cage-odour apparently reduces fighting to a minimum. However, as females are removed to individual cages to have their litters, and are then returned to the original pen, other females present will not take kindly to the newcomers, even though they may have been raised together.

It is possible to raise breeders together in one cage and when the females become pregnant for the first time, separate them from the males and return

the females, after weaning their litters, to one male only so that there are never more than one male and one female to a cage. This has been very successful as far as compatibility of the animals is concerned, but is somewhat wasteful of space, and has the disadvantage of making accurately timed matings impossible. One advantage is that the sire of each litter is known, and his performance as a satisfactory breeder can therefore be assessed.

Regardless of the methods used for breeding hamsters, one of the most important factors in maintaining a successful colony is the way in which the animals are handled by the technician. Hamsters are very quick to respond to the type of treatment they are given. A technician who is nervous about handling hamsters and therefore uses forceps or gloves will soon find himself with a nervous, vicious group of animals. On the other hand, hamsters which are handled by a person who is not afraid, who likes the animals and treats them with gentleness and firmness, will have calm, docile, easily handled animals in his colony. One can say with conviction that hamsters become what their handlers make them. Animals which are vicious can be gentled in the space of two or three days by a little attention to special handling designed to allay their fears and quiet their suspicions of humans. On the contrary, gentle hamsters can become vicious within the same short space of time if handled roughly, abruptly or with nervous hesitation. Hamsters should be scooped up in the two hands, or picked up by one hand as one would a ball, with the hand covering the back of the animal and the fingers curled under the belly for support. When the animal is released, it should be set down carefully, not dropped suddenly into its cage.

III. Diseases and Disorders

One of the great advantages of the hamster as a laboratory animal is its resistance to most diseases occurring naturally in other species such as rats and mice. Yet it has also the same advantage that these other species have, in possessing great resistance to infection from experimental surgery. There are some disorders, however, to which it is subject.

A. PARASITES

Wenrich and Saxe (1950), Saxe and Batipps (1950), Miller and Saxe (1951) have studied the intestinal protozoans of rodents. Saxe and Batipps (1950) reported that *Trichomonas muris* was found in the cheek pouches, oesophagus, stomach and intestine of hamsters, their morphology differing somewhat from region to region. The greatest population of these parasites was found in the caecum where they numbered 1,245,000 per 100 g fresh cut contents, as counted by use of a haemocytometer. Saxe (1950) successfully transferred a number of parasites to hamsters from other rodents, and the studies demon-

strated that in general the same protozoans occur in both rat and hamster, and some also in the mouse.

Wantland (1954) reported that *Giardia muris* and *Endamoeba muris* were found in hamsters; Larsh (1946) and Read (1951) reported studies on *Hymenolepis* and Larsh and Garvatt (1948) did a comparative study on guinea-pigs and hamsters, involving ascarids. We have observed pinworms (species unknown) in the caeca of hamsters and have found that they can be successfully eliminated by treating the animals with dy-piperazine as follows: mix 81·9 g dy-piperazine in 11·4 l of water. This is given as drinking water for 24 hours and the animals are then changed to clean cages. If this procedure is repeated after 10 days and once more after still another 10-day period, the animals are apparently free of pinworms, and are slow to become reinfected, provided the colony has the benefit of proper maintenance procedures.

Hamsters are subject to certain external parasites such as mites if not kept in clean, dry quarters, but are rarely infested if properly cared for. There have been laboratories where the animal rooms containing hamsters were overrun with mites, but in the same building and in adjoining rooms where care was correct, no animals were found to have mites or other external parasites upon them. This can undoubtedly be attributed to the fact that all hamsters about to be admitted to the clean rooms were dipped in a warm, 2% suspension of Aramite 15-W (an agricultural acaricide) before being put into their clean cages, and were again dipped and placed in clean cages five days later. Since the powder used in the suspension is heavy and tends to sink to the bottom of the container, the mixture must be stirred often to keep the Aramite in suspension. The animals must be completely immersed at least twice, and allowed to swim for a full minute before removing and drying. A pinch or two of wetting agent is important for the effectiveness of the dip mixture, for it allows the mixture to reach the base of the thick fur. The treatment not only kills existing mites (and some species of lice) but if done thoroughly each time, will prevent the occurrence of new infestations.

B. OTHER DISORDERS

Hamsters do not suffer from chronic respiratory infections as do rats, but sudden chilling can bring about a pneumonia-like disease which usually kills the animal in about three days.

Occasionally a pregnant female will develop paralysis of the forelimbs, but is seemingly otherwise in good health. Autopsies have revealed no obvious cause of this condition.

Although hibernation might not be considered a disorder, it is certainly not welcomed in commercial colonies, for associated with it is a reduction in breeding activity. Apparently this deep sleep comes upon animals in

colonies where temperatures fluctuate widely, especially near the floor or against the outer walls where they reach a low point in cold weather. It is important, therefore, for breeding rooms to be well insulated and held at a temperature between 72°F and 78°F (22°C and 25·5°C). Stock animals which are not being used for breeding seem to do best at temperatures of 68-72°F (20-22°C).

Perhaps the greatest mystery to hamster breeders is the exact nature and cause of a condition commonly known as wet tail. Animals young or old are subject to this disorder. They become emaciated and weak and the area about the anus becomes wet and discoloured as though the animal were suffering from diarrhoea. Autopsy has shown severe inflammation of the mucosal lining of the intestine, but no parasites, parasite eggs or enteric pathogens have been observed. It is of course possible that this disease is caused by a virus, but if this is true it is not one easily passed from animal to animal. A cage of young adult stock animals may contain six animals suffering from wet tail while the remaining six are to all appearances in perfect health.

Although there is no experimental evidence to support it, it is suggested that this disorder is precipitated by one or both of two factors: improper cage-care and inadequate diet. In one colony that showed a sudden increase in wet tail, the condition, which was caused by crowding, was cleared up in a very few days by changing the cages twice a week instead of once. In the same colony it was found that weanlings, after being separated from the mother for about three days, developed wet tail, and in this instance much evidence pointed to dietary factors. So little is known about the natural food habits of the Syrian hamster, it is not unlikely that the standard laboratory chows on which hamsters are maintained, especially in the United States, may be deficient in mineral, vitamin or other dietary components which would occur in the natural diet. The very fact that hamsters will voluntarily eat living insects is indicative of their natural attraction to this kind of food. How much analysis has been done on fresh insect tissues with an eye to their suitability as dietary ingredients? For rats and mice such considerations may not be important. For hamsters they may be of considerable importance.

Whatever the cause of wet tail, it is a constant challenge to every large-scale hamster breeder to prevent its occurrence, and it is important that its true cause or causes be discovered.

In general, hamsters require clean, dry cages for continued good health. Even a small cage, if changed often, will maintain a hamster in good condition. But a large cage, even though it has plenty of space for exercise, will produce a sick animal if urine is allowed to accumulate until the bedding is damp. Females with litters will do better if they are given shredded paper or other appropriate nesting material, and their litters will be better cared for.

Neither females with litters nor stock animals not being used for breeding should be given bedding material which will irritate or lacerate the cheek pouches. Injuries produced in this manner may lead to ulcerations. There should always be food available. There is no danger of hamsters overeating. They will store excess food and will not touch it until it is needed. Clean water is essential, especially if the daily diet does not contain fresh fruits or vegetables. The water should be changed often to prevent food particles which find their way into it from turning it sour. Spoiled food or soured water are likely to result in intestinal disturbances. Fresh fruits and vegetables should be washed thoroughly before putting in the cages, for they may have insecticides on their surfaces which are toxic to hamsters. No more fresh vegetables or fruits should be given than can be consumed in one day. Although hamsters raised in separate cages will probably fight when put together, it is also true that if hamsters are put together when weaned in groups of six or more, they will be more docile and easier to handle when they mature than animals which are isolated, one to a cage.

IV. Genetics

It is surprising that studies involving genetic factors in golden hamsters have been so slow in appearing in the literature. One reason seems to be that the occurrence of mutations has been relatively rare; and a second reason is the wide use of the animal for investigation with a general neglect of the species' own peculiarities—perhaps the greatest attention been given in England to the animal's heritable characteristics.

A. IMMUNOGENETIC STUDIES

Adams *et al.* (1956) was the first to report on the hamster's unique failure to respond immunologically to the presence of homologous skin transplants. This finding stimulated further investigation of the phenomenon and reports followed by Billingham and Hildemann (1958), Hildemann and Walford (1960) and Schöne (1961). The results of these studies show that hamsters are capable of accepting skin homografts from other, unrelated hamsters, and supporting them in good condition over a period of months. This is in striking contrast to mice and rats in which skin grafts are rejected vigorously within a period of a week unless the animals come from the same inbred line. In the case of man, cross-grafts of tissue are successful only if donor and recipient are identical twins. In short, ability to reject grafts of foreign tissue is a genetic property depending upon the presence of certain histocompatibility genes. The genes at the histocompatibility loci have been studied extensively in mice, where it has been found that both weak and strong genes may be present. When the former are in operation, rejection of skin homografts is prolonged; while the strong gene (at the H-2 locus) causes prompt destruction of the

foreign tissue. In hamsters, it has been shown that animals from certain closed colonies do not reject one another's grafts; those from certain other colonies reject grafts after some delay, and those from still other colonies reject grafts promptly, all depending upon the source of the donor graft (Billingham and Hildemann, 1958). This has led to the conclusion that in Syrian hamsters there are many weak but relatively few strong histocompatibility antigens (Hildemann and Walford, 1960). Although much speculation has arisen concerning the effect of the hamster's limited origin upon the similarity of genic content from animal to animal, no one has as yet procured present-day wild specimens for study, and Schöne (1961) points out that no heterologous skin transplantations have been made using wild *Mesocricetus*. Until our domesticated hamsters can be compared with the wild forms, we may remain in ignorance concerning certain of the effects of these many years of breeding among animals whose genic pool arose from the resources of three littermates only.

Except for these studies on the behaviour of skin homografts, few if any studies have been reported on the physiological genetics of Syrian hamsters.

B. MUTATIONS

Studies of a classical nature on inheritance in Syrian hamsters have dealt mainly with mutations affecting the colour of the coat. A brief review of the chronological order in which such mutations were recognized or reported has been given by Robinson (1960).

1947: the *panda* mutation was discovered in the United States.
1948: *ruby-eyed cream* was found in Britain.
——: *mottled-white* was reported from the United States.
1951: *British cream* was reported from Britain.
1952: the *polar* mutation was reported, but later appeared to be an animal homozygous for two separate mutations, *cream* and *white*, rather than a new mutation.
1957: *white band.*
1958: *eyeless white.*
1958: *amber.*

In addition to this listing, Robinson described the *dark-eared white* mutation in 1957 and the *brown* mutation in 1960.

Other varieties have appeared in the colonies of fanciers, but have not been formally described nor their inheritance investigated: *grey*, *tawny*, *silverblu*, *buff*, *lilac*, *pink*, *imperial* and *frost gold.*

Brief descriptions are given below of the mutations reported to date, with an indication of their method of inheritance. All have been studied in comparison to the wild type, the *golden* or *agouti*, and all except one (and possibly another) are recessive to the wild type.

1. Golden

The *golden* (or *agouti*) is the wild type described by Robinson (1955) as a

> golden brown rodent with pale cream belly fur and two characteristic throat flashes. Melanic pigment darkens the skin of the ears and is present about the genitals in both sexes (in the region of the vulva and anus in females, and in the scrotum and spots on the prepuce in males).

The eyes of *golden* animals are black, and very dark stripes of fur outline the upper edge of the light-coloured throat- or cheek-flashes. The belly fur has sometimes a distinct creamy tinge, and at other times it may be light grey. This may in itself be a genetic variation. Across the upper chest of the hamster is a continuation of the golden brown coloration of the dorsal surface, and this chest-band is almost always unbroken, though some variation in pattern exists here also. The feet, nose and tail are white. Occasionally there will be an irregular white spot on the belly surface. It is not known whether this is genetically controlled, but since similar spotting is under genetic regulation in mice, it is probably influenced in a similar manner in hamsters. The weanlings are less golden than the adults, the deep, almost orange-brown coloration increasing as the youngsters grow older and approach maturity. Young animals may also have a dark brown or black patch between the ears which generally lightens as they grow older, to become scarcely noticeable in the adult.

2. Panda

(Symbol, *s*; synonyms, *piebald*, *spotted*.) *Piebald* hamsters were studied by Foote in 1949, Foote and Foote in 1950, Orsini in 1952, again by Foote in 1955, by Whitney in 1958 and by Magalhaes in 1959. All have observed certain abnormalities which accompany the expression of this gene. Orsini reported about a 50% reduction in embryonic viability. Foote (1949) stated that no piebald hamster has a brown band on the ventral side, across the chest, as do normal hamsters. The spotting pattern is inherited as a single recessive (Orsini, 1952) and is described by Robinson (1959) as imposing semi-inviability upon the affected animal, but is not linked to other genes. It is variable in its expression (Orsini, 1952) and the amount of white present may vary from none to considerably more than 50% of the body surface. Animals bearing the homozygous recessive condition for spotting are highly nervous and require more than the usual amount of gentling to make them peaceable when handled. Whitney (1958) and Robinson (1959) reported that *piebald* and *acromelanic albinism* are neither linked to one another nor sex-linked. They may appear together in a homozygous di-hybrid condition without affecting the individual more than does either recessive mutation alone. The gene for *acromelanic albinism* is epistatic to *piebald* spotting, producing a 9 : 3 : 4 ratio in the F_2 generation (Whitney, 1958). When homozygous

piebald golden hamsters are bred to homozygous *acromelanic white* hamsters, the F_1 offspring are normal, *agouti* (*golden*) animals.

3. Dark-eared white

(Symbol, c^d; synonyms, *acromelanic albinism*, *partial albinism*.) This mutation was reported by Robinson in 1957 and again in 1959. He described the animals as having white fur, pink (pigmentless) eyes and dark (pigmented) ear pinnae. The pigment is in the skin, not in the fur. Darkly pigmented spots are also present in the skin of the prepuce and scrotum of males, and in the perineal region of females. The weanling hamsters, 21 days of age, have no pigment in the ears or elsewhere, but by 31 days of age the skin of the ears has developed a grey colour which darkens slowly with age. Robinson found that the gene is a single, recessive factor present on an autosome and inherited independently of *ruby-eye*, *cream* and *piebald* (1959). Whitney (1958) reported similar findings.

4. Cream

(Symbol, *e*; synonym: *British cream*.) The *cream* mutation was reported by Robinson (1955, 1958) to be devoid of melanic pigmentation in the fur, but retaining melanin in the skin of the ears, the eyes, the hip-gland and genitals. The gene is recessive to the wild *golden* and is not known to be linked to any other mutant gene for coat colour known at this time. The fur is a rich creamy yellow which is lighter on the belly surface. If the individual hairs are examined, it can be seen that the gene for *cream* has removed all melanin from the tip and base of the hair where it is normally found in the *golden* coat and has left two white bands in its place, apparently quite devoid of pigment. The result is a banded hair but the banding is white-yellow-white instead of the normal black-yellow-black (or brown-yellow-brown).

5. Ruby-eye

(Symbol, *ru*.) This mutant gene was also reported by Robinson (1955, 1958, 1959), who stated that it not only reduces the pigment of the eye but likewise of the fur. Both black and yellow are weakened, the black appearing blue and the yellow appearing fawn. The pigment of the skin is paler and develops later than normally in the young animal. The ears are faintly pigmented in the adult, and the genitals are flesh-pink. The new-born animals show an annulus of pigment behind the closed eyelid, and in the adult the eye is ruby-red. The animals grow less rapidly, show less vigour and have a poorer reproductive capacity than normal. There is an estimated viability of 47% among the *ruby-eye* homozygotes and this is assumed to be caused by atrophy resulting from competition between foetuses *in uteri*. The adults are described as being more nervous than are normal animals.

6. Mottled-white

Mottled-white was described by Magalhaes in 1954 and is maintained in her colony at Bucknell University. This is a sex-linked lethal mutation, recessive, which appears only in females. The affected animals have white hairs intermingled with normal coat-hairs, but the coat is thinner than normal. Some animals appear slightly greyer than normal, others are almost entirely white. The distribution of the white hairs is irregular, giving the coat a blotched or mottled appearance, and often resulting in a bilaterally dissimilar coloration. The eyes are black. These animals, as appears to be in the case in many animals bearing variations of the genes for white spotting, are smaller, less vigorous and more nervous than normal animals. When *mottled-white* females were bred to normal golden males, the resulting ratio was 229 normal males, 279 normal females and 228 *mottled-white* females.

7. White band

(Symbol, *Ba*; synonyms, *banded, belted.*) This mutation was reported by Robinson in 1960 as a new spotting mutation in the hamster. The mutation arose in the United States in 1957 and a study of its inheritance showed it to be dominant to the normal *golden*. It differs from the *piebald* which produces irregular spotting, for the white pattern produced by *white band* is confined to the trunk region and is in the form of an unbroken white band with discrete edges which encircles the trunk just posterior to the front legs. There is some variability in its expression, for in some banded golden animals, there will be scattered brown spots along the mid-dorsal line. The ears are pink at birth, but in the adult only the bases of the pinnae remain without pigment. The rest of the ear is black. Robinson found that there was more white in the coat of an animal homozygous for *Ba* than in animals that were heterozygous (*Baba*), an indication that this gene may not be completely dominant. He concluded that the gene is not sex-linked, but is a monogenic, autosomal gene. It appears to be similar in expression to the gene for *belted* (*bt*) in mice, for it produces a similar pattern. *Belted* in mice, however, is a recessive factor (Murray and Snell, 1945).

8. Light Undercoat

Light undercoat was found by Robinson and reported in 1960, but although the characteristic appeared to be familial, it occurred sporadically, and in numbers too few to use as the basis for a conclusion concerning its mode of inheritance. The affected animals possessed fur whose base was light in colour instead of grey, but this variation appears only in young animals. As they became older, successive coats became more normal in appearance until they were almost indistinguishable from coats having fur with the normal, grey base. This characteristic has also been noted in our own colony, and also without satisfactory results from mating tests.

9. Cinnamon

(Symbol, *b*; synonym, *brown*, *amber*, *gold*). Robinson reported studies on this factor in 1960. It is a monogenic, recessive, autosomal factor, producing eyes with pupils of a rich blood-red by reflected light, and ears of peach-grey. He attributes the colour of the coat to phaeomelanin, present in abundant amounts. The normal black pigment present in the golden, wild type coloration, has been changed to brown by the *cinnamon* gene, resulting in a rich cinnamon-orange colour. The normal black flashes at the cheek are changed to brown and the grey base of each hair is lighter than normal. This mutation was first discovered in the United States in 1958 by Hakes, who named it *amber gold*, and was imported to Britain early in 1959 (Beher, 1961).

10. Eyeless White

(Symbol, *WV*; synonyms: *anolphthalmic white*, *blind albino*.) Beher and Beher reported this mutation in 1959, having found it to be a partial dominant for suppression of colour. Crossing the *blind albinos* with normal animals with golden coats resulted in hybrid offspring having white ventra but otherwise normal in colour and appearance. Among fanciers these hybrids with white bellies are apparently the ones known as *imperials* and are very handsome animals. Two golden *imperials* mated together produce a 1 : 2 : 1 ratio of *blind albinos*, *imperials* and *goldens*. The *blind albinos* may be lacking eyes, or may have minute eyes which are held closed. Forcing the lids open reveals microphthalmic eyes which are pink, as would be expected in a truly albino animal having white fur and pink (pigmentless) ears. According to one breeder of these animals, the females are unable to survive the birth of their young, and only the males, therefore, are useful for breeding purposes.

The remainder of the mutations listed in the introductory paragraphs of this section have not been reported in the literature and have not been tested sufficiently to make it possible, to date, to state their mode of inheritance. Those mutations which still remain to be studied are *buff* (which may be identical with *cinnamon*); *grey* (which may be the same as what fanciers call *silverblu*); *lilac* (double recessive for *grey* and *amber*?); *frost gold* (which resembles *mottled-white* in some respects but which is not known to be sex-linked or lethal); and *pink*, which has not been seen by this author, but which is probably a variation in fancier terminology for the *cream* mutation.

Within the last year two interesting variations have arisen, one producing hamsters with a muscular degeneration appearing spontaneously in an inbred line (Whitney, 1961); and another producing weanling animals devoid of fur on the dorsal surface except for head and rump (Whitney, unpublished data). The significance of these two variations, which are apparently genetic in nature, is not yet known.

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Chapter 13

Dogs

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The dog is essentially a domesticated animal; the friend of man with a place in the human family. Pure bred animals are readily available because of the

domestic demand and the breeding of pure strains is easier than with cats. The financial advantage of a breeding colony for individual research organizations is doubtful unless the staff and accommodation are available and otherwise unoccupied. The total cost including overheads is unlikely to produce a saving and the necessity of having to produce perhaps two dozen animals within a month for, say, a toxicity programme would require a very large colony indeed. The human relationship of the dog has concentrated effort on the commercial production of efficient prophylactics against the common ailments of the animal, apart from which the dog suffers from few disorders during the first half of its life; it is therefore essentially a healthy animal, easy to breed and handle and eminently suitable for experimental work.

I. Breeds for Laboratory Use

A. MONGRELS

These animals can always be obtained. Unlike cats, they do not become unmanageable after a period of fending for themselves, and the vast majority settle down to new conditions and human attention within a week or so. They do, however, vary enormously in size, food consumption, state of health and previous contact with disease, so that they are of little value for controlled experimentation. A point in favour of the mongrel is its crossbreeding, with avoidance of genetic weakness due to inbreeding. If this point is desirable and healthy animals are available, provided variation in size and other factors do not matter, mongrels can be used for long-term experiments.

B. BEAGLES

This is the breed most commonly used in the United States. It is hardy, short-haired, moderately long-limbed and prefers to be a member of a group. Its domestic popularity in America has led to its choice for routine toxicological use; and satisfaction with the animal implies that it is capable of adaptation to isolated living quarters. Its convenient adult weight (approximately 10 kg) and uniformity of growth are added advantages.

C. SCOTTISH TERRIER

This breed has been used by us on one occasion for a chronic (2-year) toxicity experiment and has been found to have many disadvantages. It has moderately soft and long hair which needs frequent grooming, a time-consuming factor. The animal seems relatively more liable to skin conditions than many breeds. It must be kept in individual kennels because it has a marked tendency to fight, and in general it is a noisy breed. Repeated examinations of the urine have shown that in the absence of serum changes, bile pigments and occasionally bile salts will occur in the males of this species,

the females remaining relatively but not always completely free of such changes. Scottish terriers are also most susceptible to parturition abnormalities (Freak, 1962). One advantage of this breed is its uniform rate of growth with an adult weight of about 12 kg.

D. PEMBROKESHIRE CORGI

This breed is essentially a hardy working dog, originating in Wales, having a low body ideal for controlling cattle and avoiding retaliation with the hoof. The breed has become immensely popular in Britain. It is short-haired and needs little attention apart from the occasional brush and infrequent bath. It is a solitary animal and settles well in individual accommodation. It is extremely easy to handle and the old tale of its spiteful nature is, in our experience, untrue. Its growth rate is uniform with an adult weight of approximately 10 kg. It is easy to carry out routine experimental procedures with this breed; for example, if the animal becomes restless when a sample of blood is being withdrawn from the forelimb, it tends to give a sustained pull away, rather than a jerk which does not allow the operator to let the syringe ride with the leg movement.

E. SPANIEL

One of us (D. W. J.) maintained a colony of cocker spaniels for many years. The breed was used for studies in parasitology, for which purpose it is well suited. The dogs mixed well, were run in a communal exercising ground and fighting was rare. As a breed cocker spaniels are very easy to manage and handle and remarkably free from disease. Although the long ears of the spaniel predispose to otitis, this condition was not a problem in the colony.

F. COLLIE

In our limited experience with the breed, collies are not suitable for research kennels. Apart from their size, they do not appear to be suited temperamentally to the cloistered existence, do not take kindly to strangers, and are usually aggressive to other dogs.

G. BASENJI

Basenjis do not bark and for this reason they have been used in research kennels; however, they can produce a noise which is equally irritating. They are also difficult to breed and are not always easy to handle. For reference to their adaptability see Worden (1959).

II. ACCOMMODATION

Individual kennels are usually essential for the majority of animals on experiment, for the danger of fighting is ever present and inequality in the food consumption is easily possible due to different degrees of status within

the group. For the same reasons, individual runs are desirable. The best solution for keeping dogs in any number is to have a building in which there exists a central passage, kennelling at either side, and runs external to the main structure.

The details of accommodation will to a certain extent vary according to the breed and size of dog to be housed. It is proposed to consider only the small dog in future sections, since the authors feel that larger dogs are not the animals of choice for experimental purposes. This section is based on a system of which the authors have practical experience; there are of course alternatives, including that of Earl (1954).

A. SIZE OF KENNEL AND RUN

The kennel is primarily for night-time use, but must also allow for times when the animal must remain enclosed, due to inclement weather conditions or the need for constant observation. The animal must therefore have adequate room to turn about and walk a few paces in at least one direction; suggested dimensions are a width of 2 ft 3 in-2 ft 6 in (70-75 cm) and a length of 4 ft (120 cm) upwards. A certain part of the building, nearest to the work rooms, should be set aside for sick animals. Since it may well be necessary for staff to enter these compartments, a slightly greater width should be allowed. When not in use for this specific purpose, these kennels may well be used for dogs in need of constant observation or isolation.

The runs will be the same width as the kennel, if a central building is used, and should be 6-8 ft (180-240 cm) long. To obviate the isolation of the animal from its fellows, the plan designed by the United States Food and Drug Administration is well worth serious consideration. With central working facilities the kennel buildings radiate out in the form of a four-limbed cross; the terminal part of the run is bent ninety degrees inwards, so that every dog has a view of his fellows. If the kennel buildings are arranged parallel to one another, this innovation will not be necessary for, if an openwork gate is set up at the end of the runs, the animals facing other buildings will see their fellows.

Compounds for greater exercise are excellent when experimental conditions allow. An attendant should always be present during such periods, to prevent battle taking place. Short irregular periods of freedom are unlikely to improve health by exercise alone, but may well increase mental health by relieving boredom caused by routine.

B. CONSTRUCTION

Solid kennel floors are advisable, sloped away from the midline of the building, so that kennel cleaning is easily carried out. A clear pathway for liquid to flow from the kennel to an outside gulley is advisable, with the minimum number of obstructions or small corners in which dirt may collect.

The floor may be of concrete, the surface of which has been treated to be impervious to water, or it may be overlaid with plastic sheeting (the joints of which must be sealed at all points) or liquid plastic. Whatever is used, depending on the climatic conditions, the floor is likely to be relatively cold unless underfloor heating is used. In most cases therefore a bed area is also necessary. This may be made of hardwood hinged or otherwise supported some inches above the floor. It should be at least $\frac{3}{4}$ in (18 mm) thick, and can be surfaced with a material (such as hard plastic) which will not reduce the relative warmth but will deter the animal from chewing at the woodwork. In some kennels newspaper is found to be a good bedding material.

Exposed woodwork is to be avoided where possible owing to the tendency of the dogs to chew it. The partition must therefore be of metal, concrete or plastic and rise to a height over which the animals cannot jump (a minimum of about 3 ft 6 in (110 cm)). The internal gates should be opaque to reduce noise at night-time precipitated by the sight of other animals moving. The trap door to the run, the full width of the kennel, should also be opaque and set in by means of a pulley system, so that it may be opened and closed from the central passageway of the building. The only additional kennel furniture needed is the feeding utensils.

The runs are best based on concrete, divided by partitions of the same height, as in the kennels. These partitions should be solid at the base to prevent excremental contamination between runs, but the upper part may well be of rust-proof metal lattice work. The terminal gate should allow the dog a free view of its surroundings.

C. TEMPERATURE CONTROL AND LIGHTING

Underfloor heating is ideal in theory, but in practice it often has the disadvantage of a marked time-lag in response to changes in the external temperature. If the building is soundly constructed and insulated, a bed platform is provided and the connections of kennels to run are draughtproof, then in temperate climates little additional heating is necessary throughout most of the year. A minimum temperature of 7°C (45°F) is acceptable, and this may easily be supplied by radiant heat panels, out of reach of the animals.

Insulation of the building should be thorough, involving walls and ceilings. Cavity brick walls, internal foamed slats, or simple walls containing glass wool, are all efficient but vary in cost. It should be remembered that the insulation has three functions to perform, namely, to keep out the cold in winter, to keep out excessive heat in summer and to act as a sound insulator. Sick animals need a more constant and warmer environment than those in good health, so that their quarters should be separately insulated from the general quarters and maintained at a higher minimum temperature (15°C, 60°F).

Windows should be restricted to the walls of the main building; if they are

provided in the roof they increase heat loss in winter. Strip lights down the central corridor should be available to provide additional lighting for detailed examination of the animals, note-taking and accurate dosing.

D. NOISE

In periods of excitement, dogs express their emotion by barking. Their pleasure in the first appearance of the attendant each day, the anticipation of food and personal contact during cleaning, almost invariably initiate a period of barking, and this is accepted as natural by attendants who frequently become unaware of the volume of sound. Nearby householders however are not so understanding, and the prevailing wind will carry sound for considerable distances. It is advisable to bear this in mind when a kennel building is in the planning stage; positioning of the building is of first consideration. Heat insulation of central buildings should also be noise insulation, and external baffles in the form of other buildings, hedges and walls should be used to the maximum extent. A debarking operation has been described, but the results are not always satisfactory. In general, complaints of noise are usually caused by barking at night or during weekends. Any interference with the dogs during these periods should be limited to the absolute minimum. Barking outbreaks are often initiated by individual animals which give tongue on the least provocation and thereby rouse the entire colony. Such animals may have to be isolated, or removed completely from the kennels. Persistent night barkers can sometimes be quieted by pairing them with another dog. Bitches in oestrus which are kept among dogs may also cause restlessness in the colony and such animals should be kept in separate buildings if possible.

III. FEEDING

The dog is descended from carnivorous stock and many dogs will hunt, catch and devour prey; sheep worrying is but an extension of this form of behaviour. There is reason to believe that some satisfaction of ancestral feeding behaviour may be beneficial. Captive wolves often go through the motion of killing or tearing up their food—sometimes after the meal. Even the most dignified domestic dog is attracted by situations involving some measure of natural feeding habits, from chasing of chickens or other prospective prey to the digging up and gnawing of bones.

Jaw size and shape have been considerably modified by selective breeding, but the dentition of the dog remains suited to dealing with flesh. The stomach and intestines remain simple in structure and, as Abrams (1962) has emphasized, they do not possess the sacs or diverticula in which quantities of food may be for an appreciable period of time in contact with large numbers of bacteria and other micro-organisms. The average percentage relative capacities of the alimentary tract of the dog have been given as 62·3 for the

stomach, 23·3 for the small intestine, 1·3 for the caecum and 13·1 for the colon and rectum; so that the small intestine is relatively much shorter than in man.

Sound localization in the dog is about twice as accurate as in man, and odour detection is even more acutely developed; and this has undoubted significance in relation to food habits. In many instances a dog will not even bother to cross the room to taste food—virtually odourless to its owner or attendant—that it rejects by smell-association. Kalmus (1955) has shown that the capacity of the dog to detect the individual odour of a human being is quite remarkable, even when mixed with another person's odour or with some strong smelling substance. In tracking experiments, dogs have been able to distinguish between the odours of identical human twin sisters.

The group factor is of significance; puppies that have been used to feeding together will take more food in the presence of their fellows than when fed separately. On the other hand those that have been fed separately, even though reared together otherwise, do not for some time consume more food when fed together.

Exercise is a factor that may vary considerably under experimental conditions. The additional caloric utilization of running or walking outside the confines of a cage is probably a good deal lower than might be supposed from superficial observation of dogs at play. Abrams (1962) states that something like one hour's hard exercise daily is needed to dissipate the energy furnished by a dietary surplus of 15 to 20% above maintenance provision. Exercise nevertheless contributes to muscle tone, and in wild carnivores is normally an essential to capture of food. It is likely that in the wild the search for prey is, outside the breeding season, a major factor in determining the amount and degree of exercise taken. Captive or domesticated animals that are given their food regularly without any effort on their own part will tend to take relatively little exercise, although with domestic dogs there is considerable individual variation. Most owners are aware of the way in which their pet dogs relish the daily walk.

A. TOTAL FOOD REQUIREMENT

Tables of the total food requirement of the dog have been published, based upon calculated surface areas or observed voluntary intake. There is a wide individual variation in the latter, as exemplified in the studies of McCay (1949) and his co-workers on alsatians (or German shepherds), salukis and basset hounds. In these, it averaged 1 lb food per 20 lb body weight daily, and was independent of breed. Dogs having the largest appetites were the most active, but age and activity appeared to have little correlation, as did weight and food intake within the range observed. After 20 weeks, on a diet supplying 1 lb food per 36 lb body weight daily, all animals were in good condition and appeared to have received adequate food for maintenance

purposes. The digestive efficiency at this level of feeding was about 80%. In further studies on beagles, ranging from 3 months to 12 years old, it again appeared that the dry matter of the ration was digested equally efficiently at the different ages. Young puppies, however, digested the protein of a low-protein ration, and the fat of a high-fat ration, less efficiently than adults.

In adult dogs of small to medium size, such as are commonly employed for experimental purposes, the basal metabolism has been estimated to be 2 calories per kg per h (13·5 kg body weight animal), i.e. 48 calories per kg daily. The total energy requirements of the same animal would be about 80 calories per kg daily. Adult dogs on purified rations receiving 100 calories per kg daily have shown a tendency to gain in weight. From a consideration of body surface, it has been calculated that an adult dog weighing only 1 kg should receive 141 calories daily, whereas an adult dog weighing 50 kg would maintain itself on 49 calories per kg body weight daily. These requirements are of course increased during growth, gestation and lactation. Lactating bitches have an energy requirement about double that of resting females.

B. PROTEIN: CALORIE RATIO AND PROTEIN QUALITY

Whatever the type of ration fed, it is important that the ratio of digestible calories to available protein should be satisfactory and that the protein should be of good quality. Puppies that were fed casein as the sole dietary protein were found to retain somewhat less nitrogen than those fed whole egg protein, but more than twice as much as those fed wheat gluten. Nevertheless, animals on all three sources of protein gained in body weight at about the same rate, the increase per g nitrogen intake being 9·0 g for whole egg, 9·8 g for casein and 8·8 g for wheat gluten. There were, however, notable differences in behaviour, appearance and body composition. The animals fed wheat gluten were low in body protein but high in fat stores compared with those fed on egg or casein. Puppies fed egg protein were lean and active, whereas those fed wheat gluten were obese and more lethargic. A proper balance between fat stores and lean body mass is lost if the protein is of low quality or the ratio of calories to protein is unsatisfactory. In studies on adult fox terriers receiving a constant casein nitrogen intake, it has been shown that, with even a slight reduction of calorie intake, there is an increase in the excretion of urinary nitrogen. There is not, however, any change in the nitrogen balance index of the dietary protein, so that there is an increased protein catabolism. Continued restricted feeding of dietary calories will lead to severe and often dangerous depletion of the protein stores of the body.

In our own studies on diets for sledge dogs, preparatory to the Trans-Antarctic Expedition of 1957-58, we found evidence of faulty utilization of beef pemmican, a dried and concentrated food made mainly from beef and consisting of about two-thirds protein and one-third fat. The analysis of the faeces from huskies in the Antarctic indicated that about one-third of the

protein and about 6% of the fat were excreted in the faeces. Studies in metabolism cages showed that during pemmican feeding there were abnormal urinary changes, retention of water, indicanuria, and excretion of fluorescent substances. There was reduction also in the output of thiamine and of riboflavine. The animals themselves sometimes lost weight and tended to develop loose stools. Substantial improvements, in the laboratory and in the field, were obtained when the protein : calorie ratio was adjusted. A satisfactory ratio was a protein intake of 25% by weight of the ration to 2,800 calories per lb of diet. The protein intake in laboratory dogs will be lower, 15-20% or less, and to avoid nitrogen loss, there must be a corresponding reduction in the number of calories in the diet; for example, only about 1,400 calories per lb should be given if the protein intake is as low as 12·5%.

Protein quality may be affected by storage, or by damage during preparation, even when the raw material is satisfactory. In a study reported a few years ago it was found that a dog food containing fish, whale meat, meat-and-liver meal and biscuit waste, with propyl gallate as anti-oxidant, supported good growth in weanling rats after it had been dried in air temperatures below 140°C. The same diet, after drying at 140°C or 150°C, produced fluctuating gains, and after drying at 160°C led to anorexia, stunted growth, poor coats and reproductive failure. These symptoms were thought to resemble those shown by rats fed diets deficient in protein or in amino acids such as tryptophan. There was some reduction in thiamine and vitamin A in the diet dried at 160°C, as judged by chemical analyses, and possibly of other vitamins also, although the symptoms did not resemble those of any specific vitamin deficiency. The authors believed that the poor results, at least in the diet dried at 140°C, were due principally to failure to digest the protein that had become altered at this relatively low temperature by the formation of N-glycosides in the presence of reducing sugars.

Damage to, or inactivation of, amino acids through processing is, of course, a widely recognized phenomenon, and in the case of many proteins it is the available lysine that becomes reduced through coupling of the NH_2 group to form an amino sugar. Special methods are now available for the chemical determination of available, as opposed to total, lysine. The last figure may be misleading and give a faulty index of the nutritional value of the protein. Even microbiological assays, with their attendant extraction procedures, give total as opposed to available lysine. For proteins intended for feeding to growing animals, a satisfactory supply of available lysine is essential.

C. VITAMINS AND MINERALS

The individual vitamin and mineral requirements of the dog have received detailed attention, and relevant knowledge approximates that for the rat and for man. Many of the specific deficiency syndromes have been reproduced, and

in many but by no means all instances, the manufacturers of processed dog foods have taken steps to incorporate the individual ingredients, in their correct proportions, with adjustment in the form of overage or use of a stabilized preparation where the ingredient is known to be labile. Vitamin B_1 (thiamine or aneurine) is particularly liable to destruction by heat or incorrect pH, and in some canned dog foods our own studies have revealed complete loss by the time of use. This has been prevented in other instances by use of a high initial level. In our own dog and cat colonies we have found routine use of a vitamin supplement to be of protective value, and certainly the lustre on the animals' coats has been better when this has been given.

The major mineral balance of the diet of carnivores is particularly important. The work on cats of Dr. Scott and her colleagues of the Department of Physiology, Royal Free Hospital School of Medicine, London, applies very largely to dogs also, and dogs brought up on a diet composed exclusively of lean meat—with its meagre calcium content and low calcium : phosphorous ratio—will develop osteoporosis. The condition that has been described as *osteogenesis imperfecta* in young dogs is almost certainly this, and is of dietary rather than genetic origin. An excess of calcium increases the demand for vitamin D and the dog develops rickets. Adjustment of calcium intake is a simple means of preventing such disorders. In adult dogs, a daily calcium intake approaching 100 mg per kg body weight is necessary in order to maintain positive calcium balance, and a calcium : phosphorus ratio of 1·2 : 1 is the most satisfactory.

D. PRACTICAL DIETS

Adult dogs are usually fed twice daily; they should be given the main meal in the late afternoon and a light meal in the morning. A bowl of clean water should always be available. With the development during recent years of formulated dry dog foods intended to provide all dietary essentials other than water, there are now three principal ways of feeding experimental dogs. The first comprises a mixed ration, consisting of some form of meal together with one or more of such ingredients as dog biscuits, bread or other cereal products, potatoes, green vegetables and milk or milk products. From these, in combination with suitable supplements, it is possible to devise a diet that will maintain a colony in health for many years. For some years we employed with success a basal diet composed of meat, brown bread, milk and supplements. For experimental colonies, it is important to ascertain that such rations do in fact provide the necessary dietary essentials and that these are not being inactivated or destroyed.

The second way is based upon the use of formulated canned diets. We ourselves have not had much success in maintaining dogs in good condition for any length of time on exclusively canned rations, but at the Canine Health Centre, Newmarket, such a diet has given consistently healthy specimens over

three successive generations to date. A common practice is to use canned foods as part of a mixed ration, or for weekend and holiday feeding. Details of many of the current canned dog and cat foods available in Great Britain, with accompanying analytical and biological data, were given in 1961 by the publication *Which*?. The majority of such diets comprise meat, whalemeat, or fish and cereal, but some are essentially lean meat. Particular care is necessary with the latter variety to ensure that the calcium intake is adequate.

Complete dry diets, or dog chows as they are sometimes known in the U.S.A., are of comparatively recent origin in Great Britain, but so far we have been unable to maintain experimental dogs in good condition on them for any length of time. Experience in the United States is different, although some laboratories there do supplement such rations in various ways, including the use once or twice weekly of fresh meat. Dry diets would seem to offer the best potential means of maintaining dogs on, for example, a chronic toxicity experiment. It is to be hoped that further developments will extend the scope of this and similar applications.

E. WATER

Over 30 years ago Kleitman (1927) reported that there was great variation in the quantity of water consumed by individual laboratory dogs from day to day. The animals in question were not normally fed on Sundays, and on these days their water consumption was small. When the dogs were deprived of food for a number of days in succession, they drank more water daily than on foodless Sundays, but appreciably less than during days of normal feeding.

From the data in Kleitman's paper it may be calculated that, for dogs weighing 8-14 kg (17-31 lb)—within which range consumption did not appear to be related to body weight—the mean consumption on weekdays averaged 352 ml, that on Sundays only 64 ml, and that during periods of fast 148 ml. The total water intake by the body was of course even lower during fasting, owing to lack of water obtained from the food itself, and Kleitman calculated it as from one-fifth to one-third of the normal. He believed that the marked decrease in the water intake in starvation was probably one of the causes of the gradual deterioration of the salivary conditioned reflex observed in that condition.

In very hot weather, especially out of the shade, water consumption may be doubled. According to Tarbin (1955), dogs consume larger volumes of water daily at higher environmental temperatures by taking more frequent drinks rather than by any change in the amount of water taken at each drink.

A given dog appears to favour a characteristic size of drink that is not markedly affected by total food or water intake. Adult dogs with kidney damage, presumably of the chronic interstitial type, will however consume very long single drinks.

IV. Breeding Cycle

The dog reaches sexual maturity at 9-15 months and may then be used occasionally for stud purposes. After the age of 2 years dogs are generally allowed fifty matings per year.

The bitch first comes into oestrus when 9-18 months old, and thereafter every 5-8 months. Mating may be permitted at the second and subsequent oestrous cycles but is not recommended at the first oestrus. Each oestrus lasts 18-24 days, and ovulation takes place once during this period, usually within 9-14 days of the start of oestrus. Pregnancy does not affect the regular occurrence of the oestrous cycles and the bitch may be mated the next time she is in oestrus. As the bitch ages her oestrous cycles become shorter and less pronounced but she can be mated successfully up to the age of 10 years or more. However, a bitch will seldom be successfully mated for the first time after she reaches 5 years.

To diagnose the stage of oestrus, vaginal smears can be collected, stained and examined microscopically; the presence of large numbers of non-nucleated cells (as opposed to nucleated cells) is considered to indicate the time for mating. Clinically the bitch in oestrus is restless, urinates more frequently and the vulva swells and emits a blood-stained discharge. The change in the nature of this discharge from blood-stained to clear is also taken to indicate that the bitch is ready for mating. Two matings at 48-h intervals are recommended; some prefer a third mating after a similar time interval. A successful mating, particularly in young animals, can be difficult to manage, and is influenced by psychological factors. Usually the bitch is at fault in these cases and the administration of a sedative during this period is sometimes helpful.

The duration of pregnancy is 62-67 days, estimated from the first day of oestrus. A diagnosis of pregnancy can be made by an experienced person at 24-32 days after conception, by palpation of the foetuses. In general the pregnant bitch enjoys good health, and complications during pregnancy are rare. Sometimes smaller animals experience discomfort at about the 35th day due to a change in the position of the uterus, and such animals will refuse food and grunt when lying down (Freak, 1962). During the later stages of pregnancy feeding should be increased in frequency and quantity; the former is needed as the developing puppies press upon the abdominal viscera and prevent full distension of the stomach. Pregnant and lactating bitches should be given three to four meals daily.

During the last week of pregnancy the bitch commences bed making and her body temperature falls to 37·7°C (100°F). Parturition in the bitch consists basically of three phases, although in practice stages two and three occur simultaneously since the bitch produces a number of puppies. The first phase, which may last 36 h, covers the dilatation of the cervix; the bitch is restless, continues bed making, pants and sometimes vomits. There is a transient 2°F

fall in body temperature. The second stage is recognized by the appearance of straining and release of amniotic fluid and the first puppy. Straining for more than 6 h is usually indicative of dystocia. The third stage is the expulsion of the foetal membranes and occurs 5-15 min after the birth of the first puppy. The next puppy should be delivered from within 10 min to 2 h; an extension of this time is abnormal. Large bitches sometimes have a quiescent period of 3-4 h in the middle of delivering a large litter (Freak, 1962).

The average litter size depends on the breed; four to six puppies may be expected from small to medium sized bitches and up to ten puppies in larger breeds. First litters are sometimes smaller than subsequent litters. Any supernumerary claws (dew claws) on the hind legs should be removed from the puppies, and the tails docked where necessary, within their first week of life. The weaning of the puppies should commence when they are 6-7 weeks old and should be completed at the 10th-11th week. After weaning, puppies should be given four feeds per day; these are reduced to two by the time the animals are 4-6 months old.

V. Diseases

By its general behaviour, the dog can reveal much information to the trained observer on the state of its health. The dog which is in pain assumes an abnormal posture and shows changes in the pattern of its behaviour and, sometimes, its temperament. Skin lesions are licked, scratched or bitten, and thereby made obvious. The shaking of the head or the holding of one ear low indicates an infection, irritation or foreign body in the ear or ears, and pain in the leg causes lameness. Ill-health in dogs kept in kennels for research purposes is usually first indicated by a lowered food intake. Since most dogs in this category are young animals with good appetites, any dog which refuses its food should be examined promptly.

In later life, after the age of 5 years, the dog is subject to neoplasia, endocrine dysfunctions and degenerative changes in kidneys, liver and other organs.

A. THE EXAMINATION

When examining a dog it is best to have a routine which is strictly followed. The dog should be removed from its kennel and placed on a table under good light. The temperature is taken, and in a healthy dog this should be 38·3-38·8°C (101-102°F), although in puppies it may be as high as 39°C (102·5°F). At this time the consistency of the faeces can be noted. While waiting for the temperature to be recorded, the breathing of the dog should be observed and the heart beat counted. The respiration rate varies from eighteen a minute in small animals to twelve in large. The heart beat ranges from seventy to ninety a minute and is again faster in the small dog. The nervous or excitable

dog will, however, show an increased heart beat and in hot weather a normal dog may pant at 100 respirations a minute. Panting, under other circumstances, may indicate respiratory distress or heart dysfunction.

The head, nose, mouth and tonsils should then be examined. Older dogs develop deposits of tartar along the gingival border of the tooth; these deposits should be removed. The eyes should be clear and bright; a discharge from one eye usually indicates injury or the presence of a foreign body in the affected organ. Discharge from both eyes is a sign of a systemic disturbance, possibly canine distemper or infectious hepatitis. The ears should be clean although a small amount of wax is normal. Dogs with flap ears, such as spaniels, are very susceptible to ear infection since there is inadequate ventilation of the ear canal. The skin of the neck and back should be searched for parasites by removing the collar and passing the hand against the coat so that the skin can easily be seen.

The hands should then be passed over the body of the dog and down each hind leg. Any lesions or swellings and joint distentions which are not readily apparent can be detected by touch. The hind feet and the pads should be inspected carefully; ringworm sometimes takes the form of circular hairless patches at the junction of the claw and hair portion of the feet. The forelegs should be examined in the same fashion. The dog can then be clasped around the forelimbs and lifted upright to stand on its hind legs, so that the abdomen can be seen. In the male dog the sheath of the penis may show a slight soiling which is normal in some dogs, but any excessive discharge indicates an infection.

With practice, this examination of the dog can be made rapidly but without missing any gross abnormality. Practice will also make the minor abnormalities more conspicuous, and such factors as the condition of the coat, the looseness of the skin and the general reaction of the dog to the examination will aid in assessing the general health of the dog.

B. RABIES

The dog is subject to three serious viral diseases: rabies, distemper and infectious hepatitis. Rabies is an acute and fatal disease caused by a neurotropic virus. It is transmitted by the bite of a rabid animal or by allowing the saliva of such an animal to gain access to the body. Man can also become infected. Rabies has been eradicated in a number of countries, including Great Britain, Northern Ireland and Eire, but in those areas where there is a possibility of the disease occurring, any animal showing nervous signs or changes in behaviour should be considered suspect until the cause has been established. The most likely source of infection is through the purchase of stray dogs. In kennels where dogs are not introduced into the colony the appearance of rabies is unlikely, although a number of wild animals such as foxes, skunk and even bats, can become rabid and transmit the disease if they

gain contact with the dogs. The incubation period of rabies varies with site of the bite through which the infection has gained access. The minimum recorded period is 12 days; the majority of cases develop the disease within 15-25 days.

Rabies develops in two stages. The first phase consists of changes in the normal behaviour of the dog. The unaffectionate dog becomes unduly responsive and *vice versa*, and the pattern of the dog's reaction to the routine of its established life is changed. The animal may seek secluded places, become restless, bark at the least provocation and show periods of hypersensitivity. The wound through which the infection has entered is frequently scratched, rubbed or bitten; salivation is profuse. These signs last for 36 h, after which the second stage develops; this may take the form of dumb, furious or atypical rabies.

In dumb rabies, paralysis is a dominant sign and the dog is unable to keep its jaws closed and appears to have a foreign body in its throat. The pupils may constrict and squint, giving the dog a foxy expression. The barking changes in tone as the paralysis of the vocal cords progresses. Furious rabies is characterized by mania with a tendency to roam, coupled with frenzied biting of every moving object. If confined, the dog fights to free itself. The atypical form of the disease represents a combination of the furious and dumb types; the dog seeks isolation but may show periods of furious rabies. In all three forms progressive paralysis causes death 1-4 days after the onset of symptoms.

Any dog suspected of suffering from rabies should be closely confined and professional aid sought immediately. Such dogs should be kept alive, if possible, until the disease can be diagnosed without doubt.

Vaccines and sera are available for the management of in-contact cases, and for general prophylaxis. Attendants who have been in contact with a case of rabies should seek immediate medical advice as to their protection by Pasteur treatment or other method.

C. CANINE DISTEMPER COMPLEX

This is the most common viral disease of the dog; it can cause serious losses, especially in kennelled dogs, and bring investigational work to an abrupt end. Distemper is caused by a neurotropic virus, which is usually complicated by secondary bacterial infections causing intestinal and respiratory symptoms. It is very infectious.

Affected dogs develop fever and loss of appetite 7-10 days after acquiring the infection. This syndrome is often transient, lasting only a day or so, and is therefore frequently overlooked. The dog is then apparently normal for several days, after which there is a second rise of temperature which is usually associated with diarrhoea, respiratory distress, loss of appetite,

depression and a discharge from the eyes and sometimes the nose. The neurotropic effect of the virus becomes apparent sometime after the second period of fever, but may be delayed for several weeks. These signs consist of epileptiform fits, convulsions, and muscular tremors, the last of which may persist throughout life.

Young dogs may die during the intestinal or respiratory stage; older dogs are often destroyed as a result of the severity of the nervous symptoms. It has become apparent that the clinical manifestations of canine distemper vary with regard to the incidence and the severity of respiratory, intestinal and nervous disorders. Some outbreaks are associated with the rapid onset of severe nervous derangement, and include dogs of all ages. This form has been termed "hard pad", because a keratinization of the pads and nose is often present. In other outbreaks, mainly affecting younger animals, the respiratory signs dominate, while the nervous symptoms are less common. There may also be an individual variation, in that some dogs in a kennel suffer from diarrhoea, others pneumonia, and a proportion have both conditions.

All dogs must be protected against distemper, and various live and dead virus vaccines are available, as well as serum. Puppies acquire protection from the dam (assuming she is immune herself) and these maternal antibodies interfere with the immunity acquired from a vaccine. For this reason, the age at which puppies are first vaccinated is important. Too early a vaccination decreases the immunizing potency of the vaccine; too late a vaccination can leave the puppy completely unprotected. It has been shown that the bitch, through her colostrum, can protect a puppy from 3 to 9 weeks after birth. Puppies are therefore usually vaccinated at 9 to 12 weeks; however, approximately 14 days is required for the puppy to acquire its immunity from the vaccine. If infection is present in the kennels and it is desirable to ensure protection of the puppies before the 9th week, the use of serum is recommended. However, serum only imparts a passive immunity, which, although it has an immediate effect, only lasts some 10 to 14 days. Repeated injections of serum at 14-day intervals are therefore necessary until vaccination to induce an active immunity is possible.

The average pet dog probably has contact with distemper many times in the course of its life. Each challenge strengthens its immunity and for this reason the dog, once protected, is usually able to remain immune for life. Dogs in a research establishment, however, may not have this contact with live virus and therefore lose their acquired immunity. It is advisable in such cases to re-vaccinate all dogs at yearly intervals. When the attenuated live virus is used—and this is the usual biological product employed—care must be taken to ensure that the syringe is sterilized by steam or boiling water. Any contact with industrial spirit or other chemical sterilizing agent must be avoided, since it may render the vaccine impotent. Disposable syringes are particularly useful. It is likewise essential to ensure that the vaccine is not

time-expired and has been stored according to the manufacturer's instructions. These precautions may appear elementary, but their omission has resulted in serious losses through a vaccine breakdown.

It is a wise precaution to administer canine distemper immune serum in all cases of fever, especially when associated with a bilateral conjunctivitis or tonsillitis, even though a vaccination programme has been rigorously prosecuted.

D. INFECTIOUS HEPATITIS

Infectious hepatitis is a viral disease which mainly affects the liver, although it is present throughout the body during the acute phase of the disease.

A high temperature (40°C, 104°F) is the first sign, and this occurs 1 week or 10 days after the infection has been acquired. Sometimes a transient rise of temperature is the only sign of the disease. The dog becomes depressed and refuses to eat, although it will drink much water. There is a discharge from the eyes and a marked inflammation of the mouth. The abdomen is tender and there may be oedema and small haemorrhages in the subcutaneous tissue. During the period of the illness, the blood lacks the ability to coagulate and wounds incurred during this period bleed profusely. Most dogs recover from infectious hepatitis, although they take several weeks to regain their former condition. An opacity of both eyes often occurs after the severe form of the disease, but usually resolves.

Infectious hepatitis is transmitted through the urine or saliva of an infected dog. Since the live virus can be excreted in the urine of recovered dogs for many months, such animals, although themselves immune, are a source of infection. Vaccines are available and usually contain a live but attenuated virus. It is now a common practice to combine the hepatitis vaccine with a distemper vaccine so that protection against the two diseases can be given by the same injection.

E. LEPTOSPIROSIS

Two forms of leptospirosis are known in the dog; both are transmitted in the urine of infected animals.

Infected dogs are depressed, with a very tender abdomen, which is often so painful as to interfere with the gait of the animal. There is reddening of the conjunctiva and mouth, vomiting, varying degrees of diarrhoea and a marked loss of weight. There may be convulsions and a high fever. A thick discharge from the eyes is common, and the urine is scant and highly coloured.

The more serious form of leptospirosis, due to infection with *Leptospira icterohaemorrhagiae*, is spread through the urine of infected rats, and this condition, associated with jaundice, can be transmitted to man. The other form of leptospirosis, in which jaundice is not a feature, is caused by *Lepto. canicola* and is spread from dog to dog. A vaccine against both forms of leptospirosis is now available.

F. ENDOPARASITES

The puppy can be infected by ascarid worms before birth, and should therefore receive anthelminthic treatment early in its life. The established anthelminthics are still unable to do more than remove the worms in the intestinal tract. The larval ascarids move through the body cavity of the dog before becoming established in the intestinal tract; during this migration, which takes some 2 to 3 weeks, they are unaffected by any drugs and for this reason treatment should be repeated at 3- to 4-weeks intervals. Most dogs acquire an immunity to ascarids by the time they are 6 to 18 months old, but it has been shown that larval ascarids remain quiescent in the adult dog but can become active in the pregnant bitch and enter the foetus.

Dogs are also hosts to other species of worms, the most common of which is a tapeworm, *Dipylidium caninum*, acquired by eating infected fleas. The removal of the adult tapeworm from the alimentary tract of an animal is not easy, since the head of the parasite is firmly attached to the wall of the intestine. Thorough and repeated dosings with appropriate anthelminthics are often necessary. The more logical approach to the control of this tapeworm is by the eradication of the source of infection, namely fleas. However, the intermediate stages of some species of tapeworm are found in rats and mice, and in a number of food animals. The hazards of transmitting tapeworm through the medium of food animals can be overcome by a thorough cooking of fresh meat and offal. The accommodation of the dogs should also be inaccessible to rodents.

G. ECTOPARASITES

The dog is host to the flea, louse and tick. Both fleas and ticks infest the environment of the dog and merely use the animal for taking meals. In attempting control, therefore, measures must be taken to eradicate the parasite from the environment as well as from the dog. The application of any good insecticide to the coat of the dog, by wash or powder, will destroy fleas and ticks present on the dog at that time. However, the insecticidal effect is short-lived and seldom lasts more than 2 to 3 weeks. Since reinfestation can occur, any insecticidal treatment must be repeated. There must also be thorough cleaning of the kennel area, preferably with an insecticide wash.

Lice live permanently on the dog and the problem of control is that the eggs of the louse, which are attached to the hair, are not affected by insecticides. For this reason, it is usual to treat the dog again when the eggs have hatched but before the emergent lice have matured and commenced egg laying; that is at 14-day intervals.

H. RINGWORM

This disease is caused by a fungus which lives on the skin. It can be transmitted to man. Although there are several species of fungus which can cause ringworm in the dog, the most common form appears as small circular

incrustations, which have been aptly described as a round bald patch covered with cigarette ash. The ringworm often appears on the head around the eyes and on the paws, but it can grow anywhere on the body. Although ringworm is usually responsive to treatment, and will sometimes rapidly disappear without any dressing, it is contagious, especially to puppies, and affected animals should be isolated and their accommodation and utensils well disinfected.

I. ANAL GLANDS

The dog carries two anal glands, the ducts of which enter the anal rim. These sacs produce a secretion which ranges from a grey paste to brown liquid. Due to an apparent inability of the gland to empty itself or through blockage of the ducts, the anal glands become distended and in its attempts to alleviate the discomfort the dog will drag its anus along the ground. The glands can be emptied by placing one finger (preferably gloved) inside the anus and squeezing with the thumb outside. If the glands do not easily empty with reasonable pressure, excision of the gland may be necessary.

J. TOE NAILS

Overgrown or malformed toe nails are sometimes observed, especially in dogs that do not obtain much exercise. Such nails need clipping. Care should be taken to avoid the sensitive core, which can be seen if the claw is carefully examined. It is usual to cut about $\frac{1}{4}$ in (6 mm) distal to the core.

K. FIRST AID

Dogs, like all animals, are prone to wounds and general injuries. Severe injuries are uncommon among dogs maintained in good kennels, particularly if the animals are kept separate so that fighting is prevented. Severe and fatal injuries can result when more than two dogs are running together, because the pack instinct may induce all the dogs to set upon one animal. It is unusual for a two-dog fight to result in bad injuries.

Minor cuts and abrasions of the skin should be cleaned and the hair clipped from around the lesion. A topical application of a bactericidal dressing should then follow. Lotions, ointments and dusting powder each have their advocates, but the dog is likely to remove any dressing by licking. Many dogs will also remove bandages, but adhesive tapes usually remain longer. Most wounds heal quickly after cleaning, without any treatment. If it is necessary to protect a wound from mutilation by the dog, an Elizabethan collar can be fitted.

In cases of severe injury where the dog is in a state of collapse, first aid treatment should be directed towards the control of bleeding by pressure bandages, and keeping the animal warm and quiet until professional help is obtained.

L. EUTHANASIA

Dogs can normally be destroyed peacefully by the intraperitoneal injection of pentobarbitone, a special strength of which is produced commercially for this purpose.

VI. Special Examinations

From the experimental point of view alone, certain observations and tests should be carried out in order to detect departure of the animal from its normal state. We are not concerned here with the elaborate techniques and apparatus used in pharmacological studies or in surgical investigations. It is vital, however, in nutritional and toxicological work to be able to detect early changes in the animal and not merely observe the number of deaths.

A. CIRCULATORY SYSTEM

The pulse of the dog is taken at the femoral artery, which is on the inside of the thigh. Irregularities in the rhythm of the pulse are normal in the dog; this applies both to the number of the beats per minute and to the intervals between beats. Heart rate and rhythm are assessed by auscultation with a stethoscope, for which the 5th left intercostal space about the middle of the lower third of the thorax is the preferred area. In this position the first heart sound (systole) is the louder. The second heart sound (diastole) is increased in intensity by moving the stethoscope cranially and dorsally. The heart can be examined by X-ray and with an electrocardiograph using small electrodes. The electrodes are applied to the limbs above the carpal and tarsal joints (Boddie, 1962).

Blood pressure determinations can be made indirectly with a child type cuff applied to the forelimb (brachial artery) or hindlimb (femoral artery), and procedure follows that used in man. The diastolic range is 60-120 mm Hg and the systolic 100-180 mm Hg. Blood pressure measurements are difficult in small dogs, although a modified method is described by Detweiler (1959), to which authority the reader is referred for further information on the canine heart. The blood pressure can also be determined directly by a manometer attached to a needle inserted into the femoral artery.

Repeated samples of blood may be collected by venepuncture. To obtain good samples it is essential to have a good technique, for success more often depends on how the animal is held than on the ability to put a needle in a vein. Techniques differ and none is worse than another if it invariably succeeds. The cephalic vein is most commonly used but the tarsal vein is also available.

Blood may also be collected by arterial or cardiac puncture. The latter is very useful at the termination of an experiment, when 20-30 ml or even more blood may be collected with ease from the heart, whilst the animal is asleep prior to euthanasia.

B. RESPIRATORY SYSTEM

The number, rhythm and character of the respiratory movements can be assessed by observation. Percussion of the thorax can indicate lung abnormalities, as can auscultation with a stethoscope. Paracentesis, usually in the region of the seventh to eighth intercostal space, and radiography can be used to confirm suspected abnormalities.

C. DIGESTIVE SYSTEM

Observations of appetite and water intake coupled with notes on the consistency, frequency and appearance of the faeces and, where necessary, faecal tests, are the main criteria used for detecting abnormalities. Confirmatory tests include radiography, paracentesis and palpation of the abdomen; the insertion of one finger into the rectum can help to identify abdominal viscera.

D. UROGENITAL TRACT

Abdominal palpation using the finger per rectum will indicate bladder or uterine abnormalities, or pregnancy within certain periods. Confirmatory tests include the collection and examination of vaginal smears, urine tests and blood tests.

Isolated specimens of urine may be collected in simple metabolism cages, which should be about 2 ft 6 in square × 3 ft 6 in high (76 × 76 × 108 cm) and of rustless material. The floor should be of wide mesh through which the urine passes into the funnelled base with collecting bottle below. If this cage is to be used also for faecal collection, a smaller mesh floor is fixed below the wide mesh to retain the faeces. If the cage is to be used for metabolism studies, it should be large and it is essential to have a very small accessory compartment, into which the animal may put its head in order to obtain food and drink.

For more accurate work with urine, catheterization is necessary. With the bitch this can be done using a Kuschev's illuminated speculum as described by Singleton (1959). No anaesthetic is necessary. The only point to remember is that the urethral orifice lies high up the anterior vaginal wall, 1-1½ in (2·5-4 cm) from the introitus, about halfway up the vaginal length.

E. NERVOUS SYSTEM

Canine neurology and such specialized techniques as electroencephalography in the dog are still in their early stages. Radiological techniques involving the use of radio-opaque materials and air have been described by Magrath (1956).

It is frequently desirable to know the drug level in the cerebrospinal fluid in order to demonstrate passage of the blood-brain barrier. The lumbar route is not possible in the dog, so that cisternal puncture is necessary.

Following the method described by Cobb (1960), the animal is seated on a chair at a small table in a begging position. The forelimbs are fixed to the opposite legs of the table and an attendant holds the head horizontal. If the dog is in the correct position the operation is simple to perform. Using a 1 in (2·5 cm) wide-bore needle, the point of the bevel is filed away producing a shallow V-shaped bevel. The needle with its stilette is inserted in the midline at a point just above the level of a line joining the upper border of the wings of the atlas, which is easily felt. Penetration of the atlanto-occipital membrane is felt, but not entrance into the cisternal space. The needle should not go more than $\frac{1}{8}$ in (3 mm) past the membrane. When the stilette is removed, cerebrospinal fluid should flow. Rotation of the needle and abdominal pressure are two useful aids to increase the flow, which once established can be aided by very gentle suction from a syringe.

VII. Administration of Test Substances

Certain organ function tests require the administration of test substances, and these may be given either orally or by parenteral injection.

Substances can be given orally in the diet, by stomach tube or by capsule. Dietary administration is the least time consuming but also the least accurate, since the dose at any one time depends on the food consumption of the dog. This method therefore is not to be recommended and, since the dog is used experimentally in relatively small numbers, individual dosing is preferable. Passage of a stomach tube (usually a rubber catheter) is useful for giving fluids, emulsions or suspensions. Dangers however do exist; the catheter can pass into the trachea with consequent intrapulmonary injection, or the catheter may not reach the stomach and the oesophageal contents may be regurgitated and aspirated, with the same result. Administration by gelatine capsule is an easy procedure and appears to be without danger; however, it can only be used where the volume of substance is not too great.

Parenteral injection may be intravenous, intramuscular, subcutaneous or intraperitoneal. The last appears to be quite suitable in dogs and almost equivalent to the intramuscular route as regards rate of absorption for water-soluble substances.

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Chapter 14

Cats

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I. INTRODUCTION

The cat (*Felis catus L.*) is widely used in research and is the animal of choice for many studies carried out by physiologists, neuro-anatomists, psychologists and pharmacologists. Accordingly, animals are required for observations which may vary in character from acute non-recovery experiments to long-term studies of behaviour. More recently the cat has been found useful for nutritional studies and it is logical that cats are used in the study of feline diseases.

By selective breeding, many distinct breeds, races or types of cats have been developed over the years, but for laboratory use none has been shown to possess distinct advantages over the genetically heterogeneous animals which make up the bulk of the cat population. The short haired tabby, tortoise-shell, ginger, self-coloured or coloured with white, are preferred to the long-haired Persian or Angora type which require daily grooming to prevent their coats from becoming matted.

The healthy cat is a bright, alert, intelligent, inquisitive creature with bright eyes. The body is well fleshed and solid but not fat. The natural orifices should be free from discharges; the skin should not show eruptions and the

visible mucous membranes should be a clear pink colour. The animal's fur should have a glossy sheen and not harbour parasites. In adult cats, the teeth should be firmly set in the jaws and should not be unduly covered with tartar.

II. Provision of Cats for Experimental Studies

The bulk of cats used by experimentalists are purchased as and when required from dealers or from a pound. Regrettably, cats from the former source are not always free from the possibility that they may have passed through the hands of unscrupulous persons who have acquired them by theft or fraud. Certainly some cats, whether obtained from dealers or from pounds, will be incubating some disease or be heavily parasitized. Unfortunately, prolonged and careful quarantine for up to 30 days, together with attention to disease control and good feeding, do not guarantee that such animals will be free from the risk of introducing disease or parasites into already established groups. In order to avoid these hazards, some of the larger users, despite the cost involved, have set up their own breeding colonies to produce a steady output of healthy animals of the right type.

A. PURCHASE

Ideally, cats should be purchased from a single closed production colony with a known satisfactory health record; this eliminates the need for the purchaser to impose a period of quantantine. In practice, however, one is usually faced with the problems which arise when cats are purchased from a variety of sources at frequent intervals.

The user should always give a precise specification of the type of animal he requires—age, weight, sex, coat type and colour, and this information must be carefully transmitted to the supplier. When the animals are received at the laboratory, they should be carefully inspected for signs of health and for conformity to the specification. Some laboratories insist that each batch of cats received should be accompanied by a certificate from the supplier stating that the animals are his property and that he is willing to sell them for research purposes. This procedure is to be recommended. On arrival, any animal not conforming to the specification should be rejected and, after feeding and watering and a period of rest, returned to the supplier. Animals which are obviously sick present a different and often difficult problem, and it is the writer's belief that such animals should be destroyed forthwith unless the supplier will not agree, in which case expert veterinary aid should be sought.

1. Quarantine

The measures taken to prevent the introduction of disease *via* imported cats will depend to some extent on the nature of the work being undertaken in the laboratory. If, for example, cats are easily obtainable and only non-

recovery experiments are undertaken, quarantine may be dispensed with; but if the animals are to be added to groups undergoing long-term observations then rigorous isolation for a period of at least 30 days must be enforced, firstly, to ensure that the animal is not incubating an infectious disease and, secondly, to institute measures to free the animal from internal and external parasites. Since the circumstances at each laboratory tend to be unique, professional veterinary advice may have to be taken regarding the most practical quarantine procedure to be adopted. Except where cats are to be expended within a short period of time on acute experiments, vaccination against panleucopaenia is recommended. Special attention should be paid to the animal's diet, primarily to bring it up to first-class bodily condition and, secondly, to accustom it to the food it will receive in the laboratory.

B. BREEDING

The breeding of cats for laboratory use is much more expensive than obtaining them by purchase, but the additional cost may be justified, either where kittens are required for the testing of vaccines or for pharmacological studies, or where it is a great advantage to be able to add adult cats to existing experimental groups without running the risk of introducing disease. Where there is a demand for lactating or pregnant cats, an economical procedure is to purchase non-pregnant adults and to mate them as required. In these last instances the kittens are not usually reared. Such a system has been described by Dawson (1950), but where either kittens or full-grown cats are the end-product, closed colony breeding is likely to be the system of choice.

Breeding cats require a generous space allowance if good results are to be obtained and, for this reason, they should be given their freedom within rooms or large pens allowing 15 ft^2 (1·5 m^2) for each adult animal. The partitions between pens should reach from floor to ceiling. The provision of an outside run is a debatable advantage. Whilst it is true that cats enjoy sunning themselves, a solarium may attract cats from the surrounding area and these may be instrumental in introducing disease into the colony. The cost of preventing such intruders from reaching a solarium may be considerable. A satisfactory compromise may be to arrange for small south-facing windows in each room so sited that cats may lie either in the shade or in the sun.

Satisfactory cat breeding colonies may be established in existing rooms within a building (Dawson, 1950; Dickinson and Scott, 1956; Scott, da Silva and Lloyd-Jacob, 1957), in a wooden shed (Short and Lamotte, 1958) or in prefabricated concrete buildings (Paterson and Cox, 1963), but it cannot be stressed too frequently that the location chosen for breeding should be one which is removed from populations of domestic cats. Animal technicians who keep cats at home are a potential danger to closed breeding

colonies and unless they undergo a thorough routine personal decontamination, completely changing their clothing each time they enter the colony, they should not be employed on this work.

The minimum structural requirements for cat breeding or holding rooms are similar to those which apply to animal rooms in general: impervious washable smooth walls and floors, adequate drainage, good natural and artificial lighting (the latter being controlled by a time-switch), thermostatically controlled heating to maintain a steady temperature of 65-70°F (18-21°C) and mechanical ventilation to provide 5-8 changes of air per h. There is a tendency to build new animal rooms without windows, but this is not recommended for cat rooms since these animals enjoy looking on the world outside. Windows should be small and of the non-opening type and if they face south or west in the northern hemisphere, it may be necessary in some locations to provide awnings, shutters or blinds to control the entry of direct sunshine into the room during high summer.

The equipment required for cat breeding can be very simple, the essentials being dirt trays, sleeping benches, parturition boxes, feeding and drinking utensils plus a few play things. Hygienically, it would be best to have everything made in metal, but cats much prefer wood and, for this reason, it is suggested that parturition boxes, sleeping benches and the surrounds for the bottomless dirty trays be made of this material (Fig. 1). For the same reason and also to provide a suitable place for cats to stretch and scratch, partitions

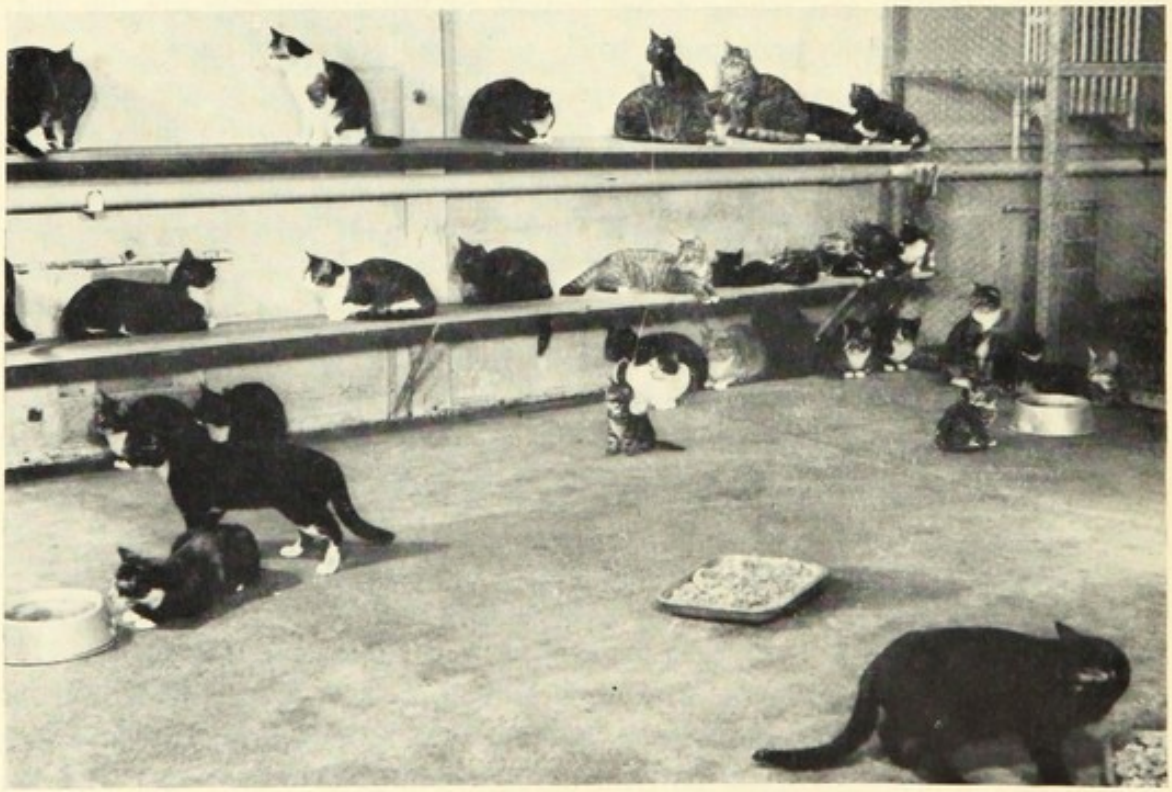

FIG. 1. Wall-type wooden sleeping benches.

may be made from 2 in × 2 in softwood battens covered with ½-in wire netting or 3 in × 1 in welded wire mesh. The bottom battens must be raised on studs ½ in clear of the floor to facilitate floor washing. In our experience, cats obtain much useful exercise climbing on wire partitions but care must be taken to bury all sharp ends of cut wires to avoid damage to the pads of the feet.

1. Practical breeding systems

The breeding system chosen will depend primarily on the objective. If matings must be observed for timed pregnancies or because the parentage must be known, as in genetic observations, then the males must be kept separately and apart from females, and the breeding may be said to be controlled. The oestrous female is taken to the quarters of the chosen male for mating. This is preferable to the reverse procedure, for males have a strong sense of territory and do not readily mate in strange surroundings. There is usually a preliminary display period which may be violent or noisy. The act of mating is of short duration; the male grasps the fur at the back of the female's neck firmly in his teeth and as soon as intromission and ejaculation have occurred he releases his hold. Generally he will retire at once to a safe distance as some females are very spiteful immediately after coitus. The female indicates that she has been successfully mated by rolling violently on the ground.

If maximum production is the objective, males and females may be run together for mating (Paterson and Cox, 1963). Contrary to expectations, these authors found it possible to have as many as four males with up to forty females in a single territory. In practice, each oestrous female was mated several times on 3, 4 or 5 successive days by several or even all of the males in turn. Fighting amongst the original males did not occur, possibly because they were all brought up together, but fresh males have been introduced from time to time, as 8-week-old kittens, and allowed to grow up within the mating area without upsetting the established males. There is an order of dominance amongst the males which is strictly in accordance with their age. Under these circumstances, the females were often observed to quarrel amongst themselves but they did not actually fight.

2. Oestrous cycle

The cat is polyoestrous, showing recurrent oestrous periods of 3-6 days every 14 days. Under the usual conditions of domesticity in the northern hemisphere, cats exhibit a period of anoestrus in the autumn and early winter and this equally applies to cats held under laboratory conditions. Scott and Lloyd-Jacob (1959) have shown that the anoestrous period may be reduced by supplementing daylight with artificial light to give a minimum light period of 12 h during the period 27 September to 15 March. These observations have

been confirmed (Paterson and Cox, 1963). During 1961, the 135 litters born in their colony were evenly spread throughout the four quarters of the year as follows:

Quarter	Litters	Weaned
1st	43	144
2nd	27	128
3rd	35	139
4th	30	113

As in other species, the stages of the oestrous cycle may be identified by the changing pattern of the types of cells which can be observed in suitably stained smears of the vaginal mucus (see Scott, *et al.* 1957). During anoestrus, small strongly basophilic epithelial cells with relatively large nuclei predominate and there are occasional leucocytes. In prooestrus the cells are larger and have marked nuclei, but during oestrus the picture is of precornified and cornified epithelial cells with small heavily stained nuclei, many of which are surrounded by a halo. During metoestrus, leucocytes appear in the smears in large numbers.

3. Pregnancy

The early diagnosis of pregnancy is best carried out by careful digital palpation of the uterus 21-28 days after mating. At that time, the embryos with their membranes and fluids are readily distinguishable in the uterine cornua as soft spheroid swellings, roughly 2 cm across. The average pregnancy lasts between 57 and 63 days.

4. Parturition and lactation

Each heavily pregnant cat should be given a large cage or pen to itself in which to litter down. If this is not done and the kittens are born within the colony, the identity of the kittens is soon lost because some mothers collect as many youngsters as they can. This leads inevitably to mis-mothering and crushing of newly born young. Mothers whose young have been stolen may suffer great pain from over-engorgement of the mammary glands; they may even develop mastitis. Another undesirable occurrence is caused by the larger kittens (5-6 weeks old) suckling heavily pregnant cats and bringing them into full milk as long as a week before they litter down.

A suitable parturition cage would be not less than 3 ft (90 cm) long, 2 ft (50 cm) deep and 1 ft 9 in (50 cm) high, but a pen with a floor double that area is to be preferred. The cat should be placed in her new quarters approximately 7 days before she is due to litter down. Cats prefer to litter down in a dimly lit area and a suitable box of either wood or cardboard should be provided for this purpose (Fig. 2). Its base should be approximately 2 ft (50 cm) long, 1 ft 3 in (40 cm) wide and not less than 1 ft 6 in (45 cm) in

height. The top must be completely closed in, preferably with a hinged lid, and the front should be open but with a sill 4-5 in deep at the bottom. The sill will prevent kittens from climbing out until they are 3-4 weeks old. The box should be placed in the cage or pen with its back to the light.

The commencement of labour is normally preceded by a period of uneasiness and food may be refused for 12-14 h beforehand. Parturition is a normal physiological process and is more likely to proceed successfully without,

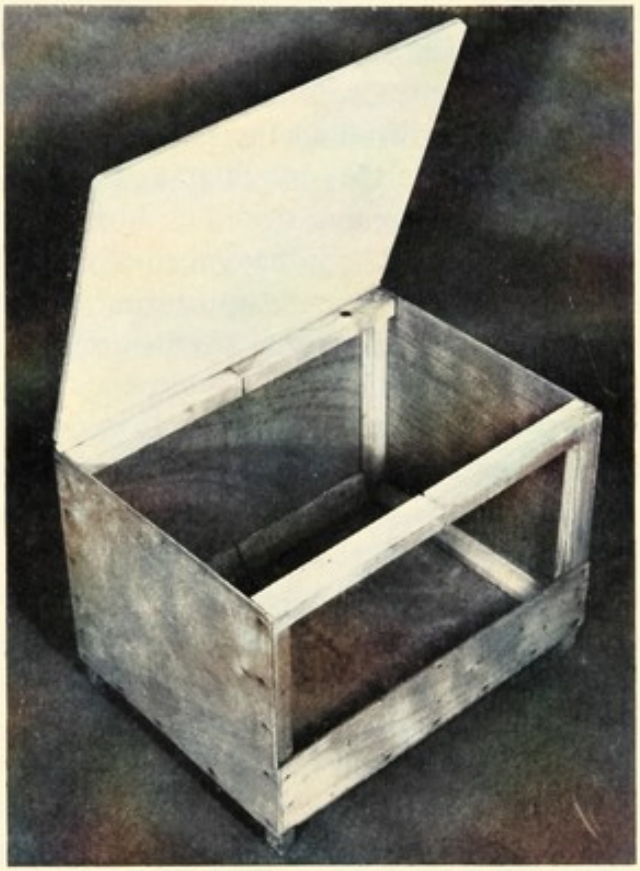

FIG. 2. Parturition box.

rather than with, human interference. The preliminary stage of labour leading up to dilatation of the cervix and the appearance of the waterbag at the vulva may take up to 4 h. The kittens are usually born within 2-3 h depending upon their number. Occasionally, dystocia or malpresentation of a foetus occurs and in such cases veterinary aid should be sought. Similarly, if the expulsion of the first foetus or foetuses has caused extreme exhaustion and several hours have elapsed without labour being resumed, treatment with a uterine stimulant under veterinary supervision will be necessary. The great majority of cats are excellent mothers and pay proper attention to their young, cleaning and drying them and consuming the membranes and placentae. Others, particularly those giving birth to their first litters, may neglect their young, in which case there is a real danger that they will die. A large proportion of

these neglected youngsters will be saved if they are cleaned, kept warm in an incubator or other warm place at a temperature of about 80°F (26°C) until such time as the mother regains her strength, when they may be restored to her.

The weight of a kitten at birth depends upon the level of nutrition in the colony, the size of the mother, the number of litters she has had and the number of young in the litter. When their eyes begin to open an average kitten will weigh about 200 g and at weaning (8-9 weeks) well-grown kittens are between 750 and 800 g. A period of steady growth continues for the next 4 months and young adult sexually mature females, 6 to 7 months old, should weigh 2·0 to 2·5 kg. As in most mammalian species, the females become sexually mature somewhat earlier than the males, which reach maturity at seven to eight months old, when they weigh approximately 2·5 to 3·0 kg.

Youngsters usually start eating and drinking during the 3rd week of life and their intake of milk from their mothers normally begins to drop during the 6th week. Weaning is usually carried out during the 8th week when the kittens are transferred to the stock runs. If entire males are required, the sexes are best separated at weaning, but if neutered male cats are acceptable, the male and female weaned kittens may be run together in age-groups, the males being castrated when they are approximately 12-14 weeks old.

5. Selection of breeding stock

The foundation stock for a new colony may be recruited from the general cat population or by obtaining a nucleus from an already established colony. A careful record of the breeding performance of each queen must be kept and replacements selected from youngsters of parents with records above the average for the colony as a whole. Short and Lamotte (1958) in the early years of establishing a colony reported a litter size of just under 3·25 and roughly 1·6 litters per queen per year. In a more established colony receiving additional artificial lighting (12-h day during the winter months), Paterson and Cox (1963) reported that during 1961 in 135 litters there were 574 live births and 35 stillbirths. Fifty kittens died during the suckling period, leaving 266 males and 258 females to be weaned. The average number weaned per litter was 3·88. The prolificity of queens of different ages was as follows:

Breeders		Litters: One	Two	Three
27	1st year	9	9	9
22	2nd year	1	9	12
8	3rd year	0	4	4
3	4th and 5th year	0	3	0
60		10 (17·0%)	25 (41·5%)	25 (41·5%)

The breeding life of the average cat should extend to 4 or 5 years, but a queen should be discarded from the breeding colony if she has not produced two litters averaging four kittens weaned during the preceding 12 months.

III. Care of Experimental Cats

Before animals are used for experimental work, each one must be carefully examined and those showing any signs of ill-health should be rejected. The normal adult cat has a pulse rate of 80-110 per min, a respiratory rate of 20-25 per min and a rectal temperature of about 100·5°F (38°C). The cat, however, is an excitable animal and healthy, normal cats may show values above those given, particularly at first examinations by strangers and in unaccustomed surroundings. The red cell count lies between 9·5 and 11·0 million per mm^3, and the white cells number between 14,000 and 18,000 per mm^3. The percentage composition of the white cell count varies from animal to animal but the neutrophils account for approximately 60%, the lymphocytes for 30%, monocytes 4%, eosinophils 5% and the basophils 1%.

Most cats may be handled with ease but a minority are spiteful and vicious. A cat understands firm but kindly handling and appreciates being spoken to and being stroked from head to tail. It normally indicates its pleasure by purring. Cats should be lifted by passing a hand over the back and under the chest. As the animal is actually lifted, the fingers should control the forelegs. The lower part of the trunk is held to the handler's body by his forearm and the hind legs of the cat hang free. Additional control may be provided by lightly grasping the loose skin at the back of the neck with the free hand. Most manipulations with adult cats are best carried out by two people, one of whom handles and controls the animal whilst the other attends to the task in hand. The danger of being scratched can be avoided by placing the cat's legs and body in a strong canvas bag fitted with a purse string which can be loosely tied round the neck.

All scratches should be immediately washed with a mild antiseptic, such as 0·5% cetrimide, and covered with a dry antiseptic dressing. At the least sign of inflammation or sepsis, medical advice should be sought. On the other hand, cat bites, however slight, should receive medical attention.

Intractable cats, usually those which have not been living in close association with humans, can present a real handling problem. One line of approach is to place the cat, or cats, in a small pen which the animal technician can enter. By talking to the cats as often as possible he will gradually win their confidence and they will allow themselves to be stroked. A few days later they may be picked up and many will become very tame.

Cats can usually be individually identified by their sex and colouring, but it is safer, particularly if the animals are running in groups, to use cat collars

bearing a numbered disc or to tattoo an identification number in the ear. In breeding colonies it is an additional advantage to tattoo the mother's number in one ear of each of her kittens while the serial number is tattooed in the other. This provides positive identification of litter-mates without having to refer continually to records. Green tattooing ink is suitable for black ears and is recommended for general use (Fig. 3).

FIG. 3. Tattooing ear marks in a kitten.

For laboratory work, cats may be confined individually in cages or they may be penned in varying numbers in a room or part of a room set aside for that purpose. The latter arrangement is labour saving but may not be advisable because of the risk of spreading infections, particularly where cats are imported from various sources. Individual cages should not be less than 3 ft (90 cm) long, 1 ft 9 in (50 cm) deep and 1 ft 6 in (45 cm) high and, if possible, a small room, pen or outside run should be available in which the caged animals may be given freedom to climb and run for one or two hours, two or three times weekly. The handling of cats during these transfers will ensure that they remain tractable at all times. Where adult cats are penned in groups and particularly when cats are added to or removed from the pen at intervals,

it is advisable to have individual sleeping quarters for each cat. One solution to this problem is shown in Figs. 4 and 5, the sleeping bench being raised 2 ft (60 cm) above floor level.

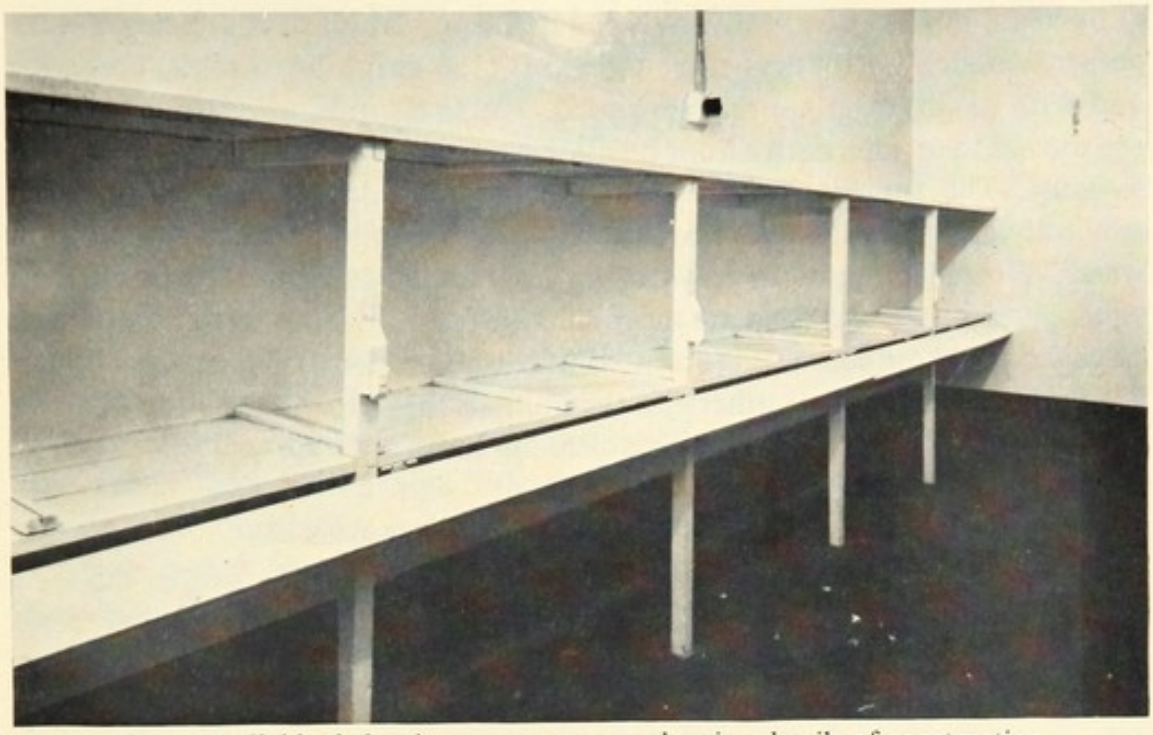

FIG. 4. Individual sleeping compartments showing details of construction.

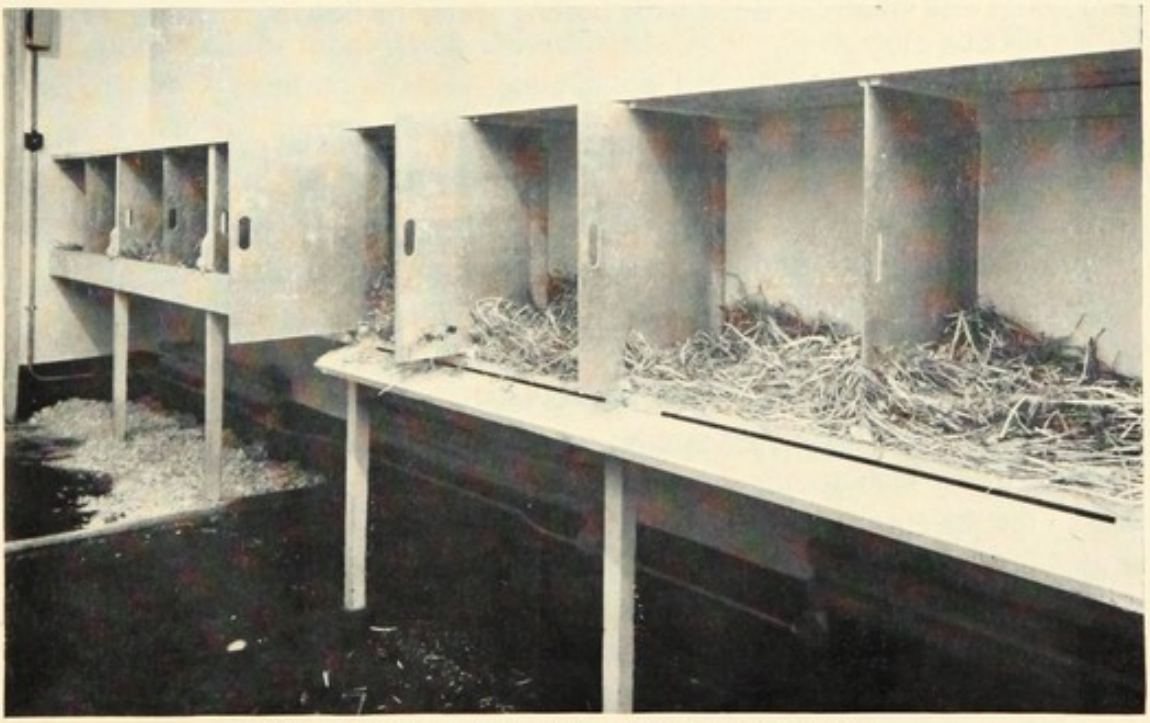

FIG. 5. Individual sleeping compartments in use.

A. EQUIPMENT

Reference has already been made to the need to provide dirt boxes. Cats are by nature clean and fastidious creatures and it is a distinct advantage to the general appearance of the colony if the dirt boxes are cleaned and filled with fresh, dry sawdust daily. Where large numbers of cats are running together, wooden bottomless surrounds, 5 ft (150 cm) long, 3 ft (90 cm) wide and 4 ft (10 cm) deep are recommended. The surrounds, after being washed, are moved to an alternative floor position each day and replenished with fresh sawdust. The fouled sawdust from the previous day should be removed promptly and the soiled floor area washed with a mild antiseptic solution in water. Welded aluminium trays of an appropriate size, say 15 in (40 cm) square and 3 in (8 cm) deep are more suitable for individual cats in small pens or cages and, here again, it is essential to wash the trays daily, and refill them with clean, dry sawdust; otherwise the animals may cease to use the dirt box and foul their cage or pen, making cleaning more tedious.

B. FEEDING AND WATERING UTENSILS

To some extent the number to be fed will determine the size of the feed utensil but, in practice, two sizes suffice. For individual cats a shallow bowl or tray, 5 in (12·5 cm) in diameter, and for groups one shallow tray, 1 ft (30 cm) square and 2 in (5 cm) deep should be allowed for feeding every fifteen cats. For milk or for water, the same dishes will suffice, but where groups are concerned one tray used as a drinking vessel should be provided for every twenty-five cats. All trays should be thoroughly cleaned each day in detergent and water and finally scalded with boiling water or flowing steam.

C. BEDDING

Dry wooden sleeping boxes or benches should always be provided. In some catteries newspaper and, in others, hay or straw is placed in the sleeping quarters, but it may not be an advantage. The presence of these materials encourages some cats, and particularly kittens, to foul their sleeping quarters, and this habit is difficult to break.

D. GENERAL METHOD OF CLEANSING CAT HOUSE

If a cat house is not to be a nuisance by causing a strong odour, then it is essential for a high standard of day-to-day cleaning to be observed. All dirt trays must be emptied, washed and replenished daily, and where this is done it may not be necessary to wash the pens down more than twice or three times a week. The frequency with which individual cages have to be cleaned will depend upon the habits of the occupant and how efficiently it uses its dirt box. At intervals not longer than once a fortnight, the walls of the cat house should be washed down or mopped with a dilute (1%) solution of washing soda in

warm water. After rinsing with clean water and allowing to dry, the walls and pens should be sprayed with a long-acting bactericidal solution innocuous to animals.

All soiled sawdust and unconsumed food should be placed in containers, preferably multiwalled paper sacks, and removed from the cat house daily and incinerated.

E. EXPERIMENTAL PROCEDURES

1. Inoculation

It is difficult to suggest a site that cannot be bitten or scratched and therefore great care must be taken to avoid sepsis. For percutaneous and intracutaneous inoculation, a site high on the shoulder may be chosen. After preliminary close clipping with scissors or a clipping machine, the area should be shaved or depilated as required.

Subcutaneous inoculations are best made in the loose tissues of the flank whilst the muscles of the thigh is the site of choice for intramuscular injections. Intravenous inoculation is not a simple technique in the cat and should only be attempted in placid, quiet animals. The radial vein in the forelimb is the most convenient vessel. Intraperitoneal injections should be given about 2 cm to the right of the navel but care must be taken not to inoculate directly into the cavity of the stomach if the cat has recently eaten, or into the bladder if this is full. Intrathoracic inoculations may be given between the sixth and seventh ribs on the right side. For injections other than intravenous, 20 s.w.g. needles 1-1½ in (2·5 cm) long are commonly used. For intravenous work, a shorter, finer needle (24 s.w.g. 1 in (2·5 cm) long) is more satisfactory.

Sites selected for inoculation should be clipped with scissors and drenched with antiseptic solution or ether immediately before the inoculation is carried out. All syringes and needles should be sterilized. Where batches of cats or kittens have to be inoculated, a separate sterile needle for each inoculation is recommended.

2. Collection of blood samples

Small samples for blood smears or counts may be obtained by puncture of the pad or from the marginal ear vein. Larger samples may be withdrawn by syringe from the radial vein but the use of an anticoagulant is necessary. Large amounts must be taken by careful heart puncture under general anaesthesia.

3. Surgical procedures

These must always be carried out with full sterile precautions if the subject is to be allowed to recover from the anaesthetic. The after-care of such animals must be of the highest order. Adequate nursing and a suitable diet must be provided.

In all operations, including non-recovery experiments, adequate anaesthesia must be maintained until surgical interference is finished, or death supervenes.

4. Nursing of cats after operation

Cats recovering from an anaesthetic should be placed in a cage by themselves until the effect of the anaesthetic has completely worn off and they are able to fend for themselves. The cage provided should be warm and free from draughts. Whilst the animal is still immobile, e.g., after barbiturate anaesthesia, it should be covered with a light blanket to help the animal conserve its body heat and so aid recovery. It may be an advantage to have an infra-red heater above the cage to help keep the animal warm.

IV. Feeding

Cats are carnivores by nature and even well-fed domestic cats will hunt, catch and devour many small rodents and birds. Among the common domestic and laboratory animals, the cat is perhaps the one about whose nutrition the least is known and this may be attributed to the difficulties formerly experienced in breeding cats under controlled conditions (Scott, 1960). In reviewing the subject Scott stresses the fact that satisfactory growth, maintenance of good bodily condition and successful pregnancies are closely bound up with the total daily food intake. Cats have an unusually high requirement for protein and most of the animal protein foods—meat, offal, poultry and fish—are relished. Excellent growth and satisfactory reproduction have been obtained in cats fed exclusively on meat (supplemented by mineral salts) so it is unlikely that carbohydrate itself is an essential constituent of the diet, but a high fat diet is known to be beneficial. A satisfactory diet, on a dry weight basis, should contain approximately 50% protein and 15% fat. The vitamin requirements of the cat have not yet been accurately determined but the vitamins of importance are A, D and B complex (riboflavine, thiamine, niacin, pantothenic acid, pyridoxine and folic acid). Vitamin E is essential and there will be a large requirement if the level of unsaturated fat in the diet is high. To ensure an adequate intake of these vitamins and to avoid mineral deficiencies, the use of specially prepared vitamin and trace element mixtures is to be recommended. These may be mixed with the diet in the final preparation stages. Alternatively, dried yeast and dried liver may be added at the 2% level.

Any practical system of cat feeding is usually based on freshly cooked scrap fish or meat. After thorough cooking, the protein is minced with approximately two parts by weight of cooked potatoes. This is remixed with some of the gravy from the fish or meat to produce a semi-solid hash. More of the gravy may be utilized if a high protein cereal meal is available to mix in as a drying agent. Certain proprietary complete dry dog or cat foods are especially

useful for this purpose. In many countries there is a section of the food industry which caters specially for cats, and the tinned foods produced are valuable, if expensive, alternative sources of protein.

The diet of laboratory cats will to some extent depend upon the amount of money available, on the local availability of scrap fish and meat and on the amount of labour and the facilities available on the one hand for cooking and, on the other hand, for cold storage of uncooked meat and fish.

The amount of food (on a dry weight basis) which a normal healthy adult cat will eat daily is approximately 4% of its body weight. Kittens, and pregnant and lactating mothers may consume up to twice this amount. In practice, it is the general custom to feed cats to appetite and the amount to be fed daily is left to the judgment of the technician in charge. Normal stock cats should have one main meal daily and this they should consume in about half an hour. Pregnant cats should be given a small supplementary feed either early in the day or late in the afternoon, depending upon the time at which the main meal is offered. Kittens, on the other hand, have a voracious appetite but seem easily satisfied and when first weaned should have two feeds daily. When $3\frac{1}{2}$ months old, one feed should be gradually increased and the other reduced and by 5 months only one main meal a day should be given.

Traditionally, cats are given milk to drink and there is nothing against this. However, fresh or even pasteurized milk turns sour quite quickly, especially in the summer months. A satisfactory alternative is to reconstitute full-cream dried milk with water which has been raised to at least 160°F (70°C) in sterilized utensils. This preparation will normally keep fresh for 18 h at 70°F (21°C). It has been noticed that some cats prefer a mixture of one part by weight of dried full cream milk with 12 parts of water rather than the somewhat denser product which corresponds to cows milk (one part by weight of dried milk to seven parts of water). Contrary to general belief, cats drink large quantities of water and a fresh supply of clean water should always be available.

Cats naturally eat and drink from ground level and all dishes in which food and water are offered should be shallow and placed on the floor. No advantage is gained by raising them as cats will simply drag the food out of the vessel and on to the floor.

The food offered to cats after recovery from general anaesthesia must be light and nourishing. Unless a special diet has been prescribed, milk containing a little glucose (1 teaspoonful to $\frac{1}{2}$ pint) may be offered during the first 24 hours. On the second day, boiled fish or minced cooked liver or meat may be given in addition to milk. Whatever food the cat enjoys best should form the main item of its diet for 3 or 4 days. It should then be encouraged to return to the colony's stock diet. Occasionally, after anaesthesia, a cat will refuse any of the foods it normally enjoys and under such circumstances it may readily eat unaccustomed items, e.g., beaten up raw egg, tinned cat meat or poultry.

V. Anaesthesia, Euthanasia

The choice of anaesthetic will vary with the circumstances surrounding the experiment. The possibility of using local anaesthetics which paralyse nerve fibres and nerve endings by direct action for minor surgical procedures is often overlooked. A 2 or 3% solution of procaine infiltrated into the skin, subcutaneous tissues and around the track of the regional nerve quickly produces satisfactory local anaesthesia. Adrenaline is often mixed with local anaesthetics to limit absorption and to act as a local haemostatic but this latter effect may conceal haemorrhage which should have been dealt with by ligation of torn vessels.

The choice of a general anaesthetic will depend upon the nature and duration of the surgical interference. For many purposes, ether remains the agent of choice. It may be administered with a mask using a Wright's apparatus (air pumped either through or over ether). The induction of anaesthesia with ether is a task requiring skill and it should only be undertaken after proper instruction. When the state of unconsciousness is first reached, there is usually a period of excitement and care must be taken not to release the cat at this time. Breath-holding by the subject can also be very alarming, but it rarely leads to cardiac or respiratory failure, provided administration of the anaesthetic is suspended and gentle artificial respiration is applied until regular breathing is restored. Surgical anaesthesia is reached when the corneal reflex is suppressed. Recovery from ether is rapid and the effect on foetuses is not prolonged.

Where a closed-circuit apparatus is available, cyclopropane may be used and is satisfactory. Halothane, a recently introduced volatile anaesthetic, is given in a closed-circuit apparatus.

Chloroform is not a suitable anaesthetic for cats.

The use of volatile anaesthetics is aided by premedication with atropine and, in some cases, pethidine also.

Compounds of the barbiturate group are useful where it is an advantage to have the head and neck free of masks or other equipment. For very short operations, thiopentone, and for longer operations, pentobarbitone are reliable agents. The former must be given intravenously and the cat may be kept anaesthetized for up to one hour by intermittent administration. Recovery is fairly rapid when anaesthesia has been of short duration. Although pentobarbitone is usually given by slow intravenous injection, a full anaesthetic dose, which may be effective for up to 6 h, can be given intraperitoneally. This procedure is particularly useful for timid or fractious cats. Recovery from pentobarbitone is slow. Barbiturate surgical anaesthesia may be terminated by the intravenous injection of bemegride but the full recovery time, that is to the standing position, is not greatly shortened. Bemegride may also be used as an antidote in accidental overdosage with barbiturates.

Probably the most satisfactory method for the destruction of cats is to give a lethal dose of pentobarbitone by intraperitoneal or intrathoracic injection. Special solutions containing 200 mg of pentobarbitone per ml are commercially prepared for this purpose. Care must be taken not to confuse them with the anaesthetic solutions. Animals already deeply anaesthetized may be destroyed by an overdose of the anaesthetic and more rapidly by an intracardiac injection of 2·0 ml of chloroform or simply by opening the chest cavity on both sides.

VI. Health

The maintenance of a healthy colony should be the endeavour of all concerned and any departure from normal in any cat or kitten should be reported to the colony supervisor without delay. The likelihood that infectious disease or parasitic infestations may gain a foothold in a cat house must be guarded against. It is a relatively easy task, starting with a small number of animals, to build up a closed colony of cats free from most of the troublesome diseases and parasitic infestations of this species. A few simple precautions usually ensure that the laboratory or laboratories drawing cats from this closed colony themselves enjoy freedom from intercurrent infections. Laboratories which recruit their cats from a variety of sources must wage a constant battle by quarantine and isolation to guard against the risk of introducing disease into their cat houses.

In a closed colony all kittens should be vaccinated at weaning age with a potency tested vaccine against panleucopaenia (infectious enteritis). If the animals are to be used for long-term studies or for breeding purposes, they should be re-vaccinated after each successive 12-month period. In our experience, pneumonia associated with *Bordetella bronchiseptica* infection has been troublesome in young cats. Although the disease responds to antibiotic treatment with chloramphenicol it is not easy to detect in the early stages. We therefore made an autogenous heat-killed vaccine for immunization of the breeding stock. Basic immunity is produced by two subcutaneous doses of 1·0 ml vaccine (opacity Brown tube 2) 14 days apart. Subsequently, booster doses of 1·0 ml are given annually. The disease is now seldom seen.

A form of navel-ill or joint-ill due to a haemolytic streptococcus was frequent in kittens 7-14 days old. An identical organism was found to be present in the vagina of most breeding females and it was likely that infection was conveyed to the newly born kittens either at parturition or shortly after by the mother as she cleaned herself and the youngsters. The organism is penicillin-sensitive and cases have been almost eliminated by instilling one dose of intramammary penicillin (as produced for mastitis in cattle) into the vagina of each parturient cat, 1-4 days before the commencement of parturition (Fig. 6).

Possibly the most troublesome condition in young kittens is a purulent

conjunctivitis which occurs when they are about 2-4 weeks old. The recovery rate is 100% provided the eyes are cleaned twice daily with lukewarm water and penicillin eye ointment is introduced under the lids (Fig. 7). The disease runs a 4- or 5-day course.

Fleas and lice were eradicated from the original cats by dusting them and their sleeping quarters with a powder containing 0·2% of the gamma isomer of benzene hexachloride twice, at weekly intervals. This treatment was

FIG. 6. Intravaginal instillation of penicillin.

repeated two or three times as necessary. The parasites have not reappeared. Roundworms were eliminated by treatment with one of the salts of piperazine and tapeworms have disappeared because wild mice do not form part of their diet. Otodectic mange or canker of the ear is widespread in cats. This has been brought under control, but not completely eliminated, by the use of dibutylphthalate which is highly efficient, softening the crusts and killing a high proportion of the parasites. A satisfactory routine leading to the elimination of the disease has not yet been evolved although several acaricides have been tried.

A period of quarantine of at least 28 days is recommended for cats not obtained from an associated breeding unit or a reliable closed colony source. They must be subjected to a careful clinical appraisal before acceptance. A lesser period of quarantine, say 10-14 days, may be acceptable if the cats are

not going to be introduced into an animal room containing cats already under observation. Preferably, the isolation quarters should be in a building separated from the main animal house or the rooms allocated for this purpose should be so designed that the animals can be introduced into them directly from the outside. The personnel assigned to the quarantine rooms must be able to decontaminate themselves before leaving the area.

Cats intended for long-term observations should be vaccinated against

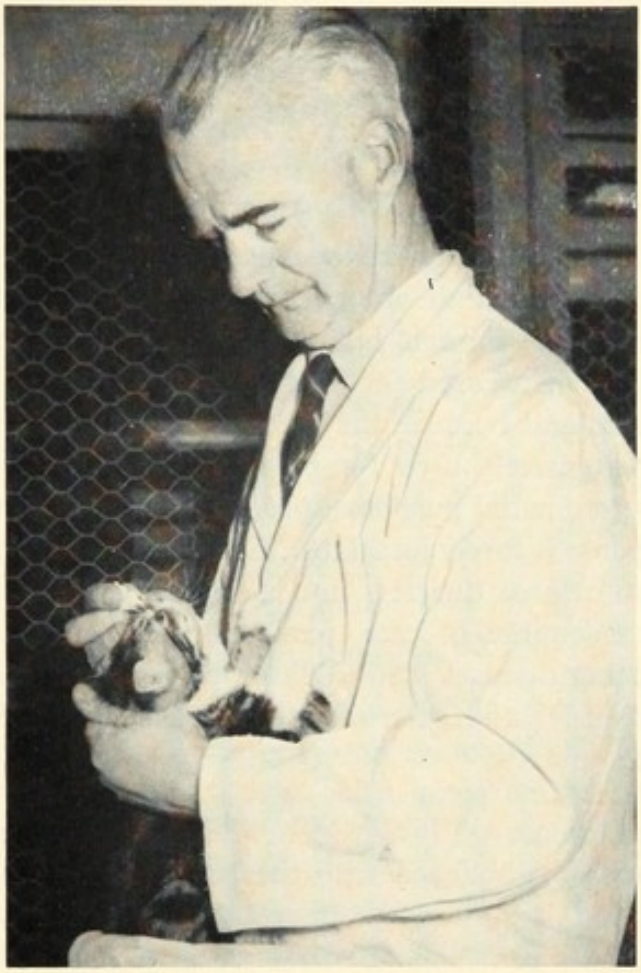

FIG. 7. Applying antibiotic ointment to a kitten's eyes.

panleucopaenia 24 h after they have entered quarantine, provided their temperatures have remained normal. They should be examined for internal and external parasites and, if necessary, the appropriate treatment should be given.

During quarantine, a careful watch should be kept for skin lesions due to ringworm. Treatment of individual animals in a colony is rarely justified, but treatment of in-contacts with griseofulvin may be useful in limiting its spread.

As a great deal of expertness is required for the differential diagnosis of cat diseases, laboratories without a veterinarian on the staff should not many hesitate to call in a veterinary surgeon when in need of expert advice.

VII. Transportation

Cats travel well either individually or in small groups. They should be sent in well constructed, ventilated wooden boxes or baskets strong enough to withstand a certain amount of rough handling. Remember that a cat which escapes whilst travelling will be difficult, if not impossible, to catch and will undoubtedly suffer much hardship before it finds itself a new home or a safe refuge. It is extremely difficult to arrange for cats on a journey to be fed and watered by strangers and, for this reason, on any journey which may last more than 10-12 h they should be accompanied. Cats should never be sent by rail or other carrier service until the sender is satisfied that the recipient is expecting the animals and will collect them promptly at the terminal point.

VIII. Summary

Cats, along with dogs and horses, because of their temperaments and intelligence, receive superior and often more individual attention than other experimental animals. Although by nature timid creatures, cats become highly co-operative when cared for by understanding and patient handlers. Because of their size and because they are good subjects for general anaesthesia, they are extensively used for physiological observations. Although rarely bred for experimental purposes, productive colonies have been established, but the expense is rarely justifiable. Cats require a certain freedom for exercise, dry, comfortable quarters and a high protein diet if they are to thrive. The major scourge of cats, panleucopaenia, can be prevented by vaccination and other diseases held in check by proper veterinary attention. Parasites, both internal and external, need not be troublesome if routine treatment is carried out.

Acknowledgement

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Chapter 15

Monkeys

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I. INTRODUCTION

In recent years the number of monkeys used by research laboratories has greatly increased, due largely to the use of these animals for the production and testing of poliomyelitis vaccines. Also, research workers in other fields have increasingly found monkeys to be useful for certain aspects of medical and dental research. The species *Macaca mulatta* (rhesus), *Macaca irus* (cynomolgus) and *Cercopithecus aethiops* (vervet), weighing between 1·5 and 5 kg, are commonly used and the methods described in this chapter apply to animals of that size. With certain obvious modifications, the techniques generally are satisfactory for larger animals including baboons (*Papio* species).

II. TRANSPORTATION

Unlike most animals which are bred in laboratory animal houses or special breeding establishments, the majority of monkeys used are caught in their natural habitat and transported, frequently thousands of miles, to the laboratory. It is desirable, therefore, that users pay some attention to the method of transportation from the country of origin, in addition to the husbandry of the animals within the laboratory.

Every possible effort should be made to reduce to a minimum any distress

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which is experienced during transit, for economic as well as humanitarian reasons. Only animals in good health, and preferably with veterinary certificates of fitness, should be transported, and the journey should be completed as quickly as possible. Careful consideration must be given to the method of packing the monkeys to ensure that overcrowding is avoided. Where journeys are likely to be prolonged it is desirable to pack animals singly or at the most in pairs so that the risks of injury due to fighting and bullying may be reduced. Pregnant animals should be packed singly.

It is essential that travelling crates are designed in such a manner that there are no projections on which the animals are likely to injure themselves. Fluctuations of temperature during a journey are likely to occur, but sudden changes should be avoided (British Standards Institution*). Where journeys are likely to be longer than 6 h, arrangements must be made for supplying water at regular intervals, not more than 6 h apart. A supply of food should be provided during a journey, although a monkey can be left comfortably for 12 to 24 h without food if it has been fed immediately before shipment. After a journey, it is desirable that they are rested and little should be done to them other than providing them with food, water and medical attention if necessary, for the first 48 h after arrival.

III. Infectious Conditions and Their Relation to Management

For humanitarian and economic reasons it is desirable that the spread of infectious diseases within a monkey colony be controlled and this can be achieved only when a high standard of hygiene is maintained within the animal house. Some diseases of laboratory primates are transmissible to man and it is therefore essential to control these so that potential hazards to animal technicians and laboratory workers, who use simian tissue, are reduced to a minimum and, if possible, eliminated.

One of the principal hazardous infectious agents is B virus (Sabin and Wright, 1934). This agent, which has been observed mainly in rhesus and cynomolgus monkeys (Keeble, 1961), is not, apparently, a serious pathogen in monkeys but has been responsible for a number of fatal cases of ascending myelitis in man (Davidson and Hummeler, 1960), mostly due to bites and contamination of wounds by infected material. It is possible, however to infect laboratory animals with aerosols of B virus (Chappell, 1960) and this must therefore be considered as a possible method by which man can be infected. This virus is immunologically related to herpes virus in man and lesions which it produces are initially vesicular. These occur mainly on the dorsal surface of the tongue and muco-epithelial border of the lips (Keeble *et al.*, 1958). (See Figs. 1 and 2).

Because of the occurrence of B virus lesions in the mouths of infected

* Although these recommendations were designed for transportation by air, much of the information, in principle, is applicable to other forms of transport.

animals it is essential that techniques of handling monkeys are adopted so that the risk of bites and contamination of workers is kept to a minimum. Newly imported monkeys with lesions on the lips or tongues should be destroyed and the carcases incinerated.

Other viruses known to be carried by monkeys have also been recovered

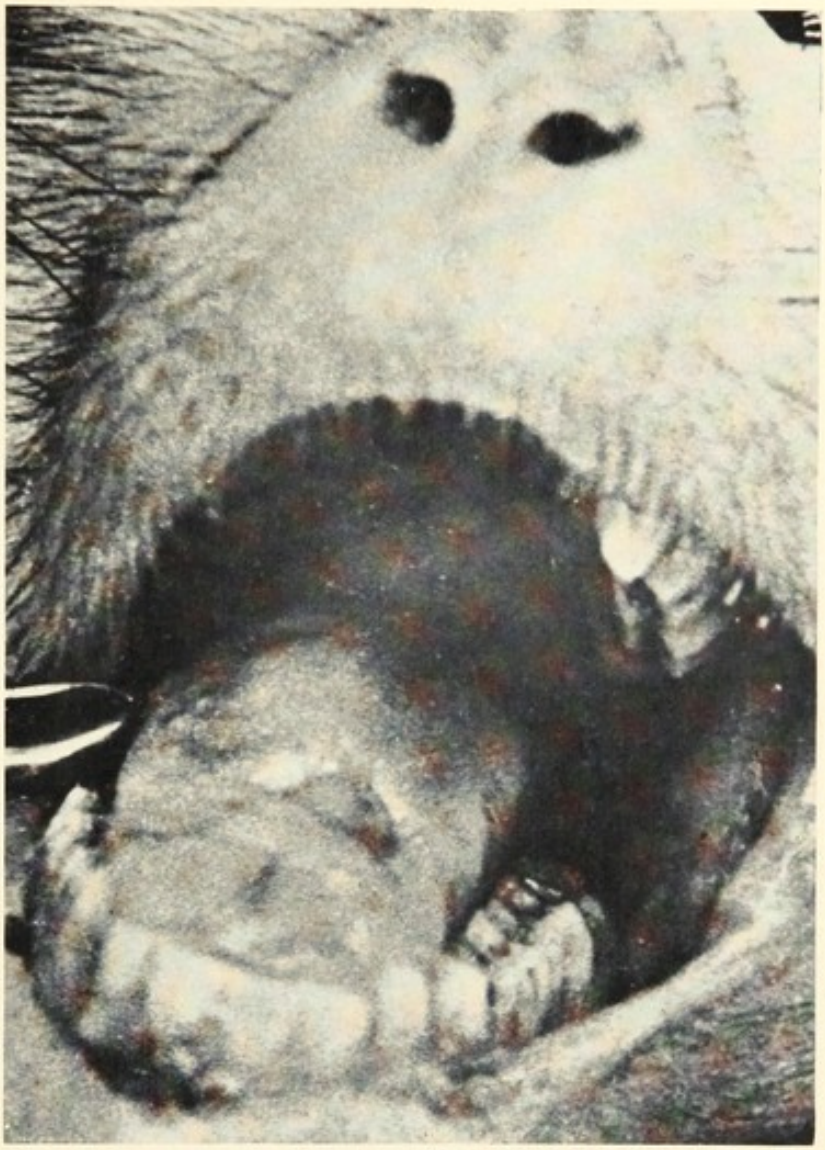

FIG. 1. B virus lesions on tongue.

from intact animals and from cell cultures prepared from their tissues (Tobin, 1960). Although some of these are not pathogenic for man and do not cause apparent disease in monkeys, their presence in tissue for laboratory purposes and vaccine preparation is obviously undesirable. The control of a number of these agents can be achieved to a considerable extent by good animal husbandry and animal house hygiene.

It is advisable not to have different species in the same room or to mix new intakes with existing stock. The former is of especial importance when the

different species are imported from different geographical areas. While the species may be kept separate in the laboratory, it must be remembered that in the transportation of monkeys from various parts of the world the species are regularly transported together in the same compartment of the aircraft, ship or other means of transport having a route which serves several areas of

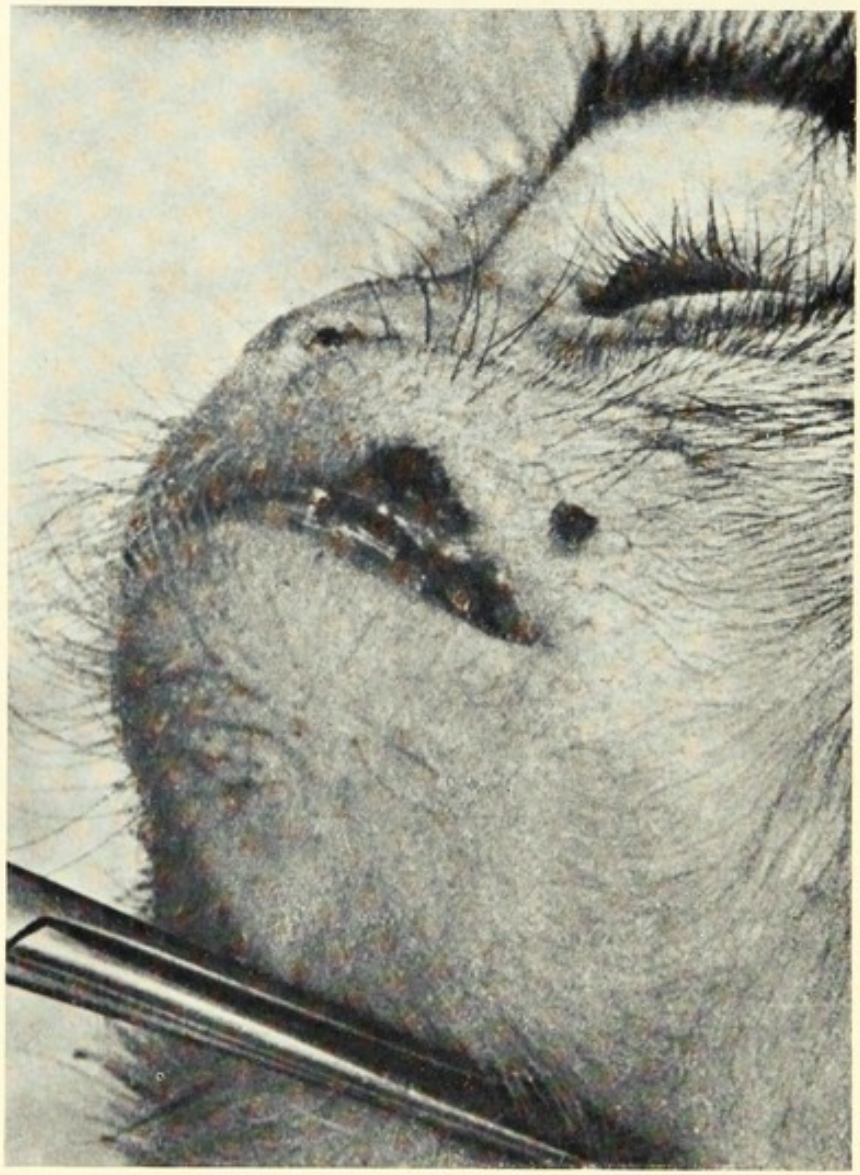

FIG. 2. B virus lesions on lip.

the world. Quarantine of new intakes is, therefore, of the greatest importance.

Only animals in good health, which have been kept in isolation for at least 6 weeks, should be used for tissue culture and during the period of quarantine not more than two animals should be kept in one cage. The principal bacterial infections which present a hazard to man as well as to monkeys are those caused by *Mycobacterium tuberculosis*, *Salmonella* and *Shigella* spp. of organisms.

Tuberculosis is a relatively common disease of monkeys in captivity, but is of low incidence in monkeys in their natural state (Fremming *et al.*, 1955). A survey of literature tabulated by Ruch (1959) showed that the causes of the disease were due mainly to mammalian and bovine types, though avian bacilli were isolated from some animals. The clinical signs of tuberculosis are not evident until the condition is quite far advanced and animals which appear in good condition may be severely affected. Tuberculin testing is of some value, but it must be noted that false positive and false negative reactions can occur. A method commonly used for this test is to inject into the upper eyelid 0·1 ml of a 1 : 10 dilution of Koch's Old Tuberculin. The test is read after intervals of 48 and 78 h. Monkeys affected with tuberculosis should be destroyed. Anti-tuberculous therapy of affected *M. mulatta* monkeys has been carried out with success (Fremming *et al.*, 1956) but such a procedure is justifiable only in very special circumstances and after very careful consideration of the possible consequences of such action by all concerned.

Salmonella and *Shigella* spp. may be isolated from a high proportion of monkeys. Habermann and Williams (1957) observed that the incidence of salmonella infection was 14·9% and that for shigella 10·2% of 275 rhesus monkeys. These organisms are sometimes associated with dysentery in monkeys, a condition which causes a high morbidity and mortality in laboratory primates. This condition does not respond readily to treatment and affected animals, if they survive, are unthrifty for a long period after the illness. A review of the different types of treatment which have been attempted is given by Ruch (1959). Efforts therefore should be made toward the prevention of dysentery by continuous observance of good hygiene, and regular prophylactic treatment with antibiotics or sulphonamides for the first week or two after the monkeys' arrival at the laboratory may be of some value.

For practical purposes it should be assumed that agents other than viruses and bacteria are potentially hazardous for man. In this category are protozoa such as *Entamœba histolytica* and intestinal helminths of *Œsophagostomum* spp. The presence of these organisms must be considered when decisions are being taken on methods of sterilization of equipment.

IV. Staff

Because of the potential hazards to animal staff and laboratory personnel, particularly when dealing with newly imported monkeys, care must be taken to ensure that unnecessary risks are reduced to a minimum. It is desirable that all staff wear a complete covering of protective clothing when handling monkeys or equipment which comes in contact with them. Suitable protective clothing consists of a boiler suit, cap, rubber boots and rubber gloves; additional protection is also given by surgical masks and goggles. It is

important that personnel are trained in the proper use and care of protective clothing. When handling monkeys which have not been anaesthetized, leather gauntlet gloves may be slipped over the rubber gloves. Preferably, staff should have a clean set of clothing daily.

It is essential that animal technicians are physically fit, and as a protection against tuberculosis, should receive BCG vaccine if necessary. Regular medical examination of staff is desirable. Eating, drinking and smoking must not be allowed within the animal house.

V. Housing

The type of building, general design and requirements necessary for keeping monkeys need not differ greatly from those used for other species of laboratory animals. Occasionally, however, monkeys will escape within the building and special additional care must be taken to ensure that they cannot escape outside. It may be desirable, therefore, to provide each room, or a set of rooms leading off one corridor, with two doors so that one may be closed before the second is opened. Care must also be taken to ensure that if animals do escape, there are no features which could injure them and by which they could escape, e.g. exposed lift shafts or ventilating shafts. Because of the

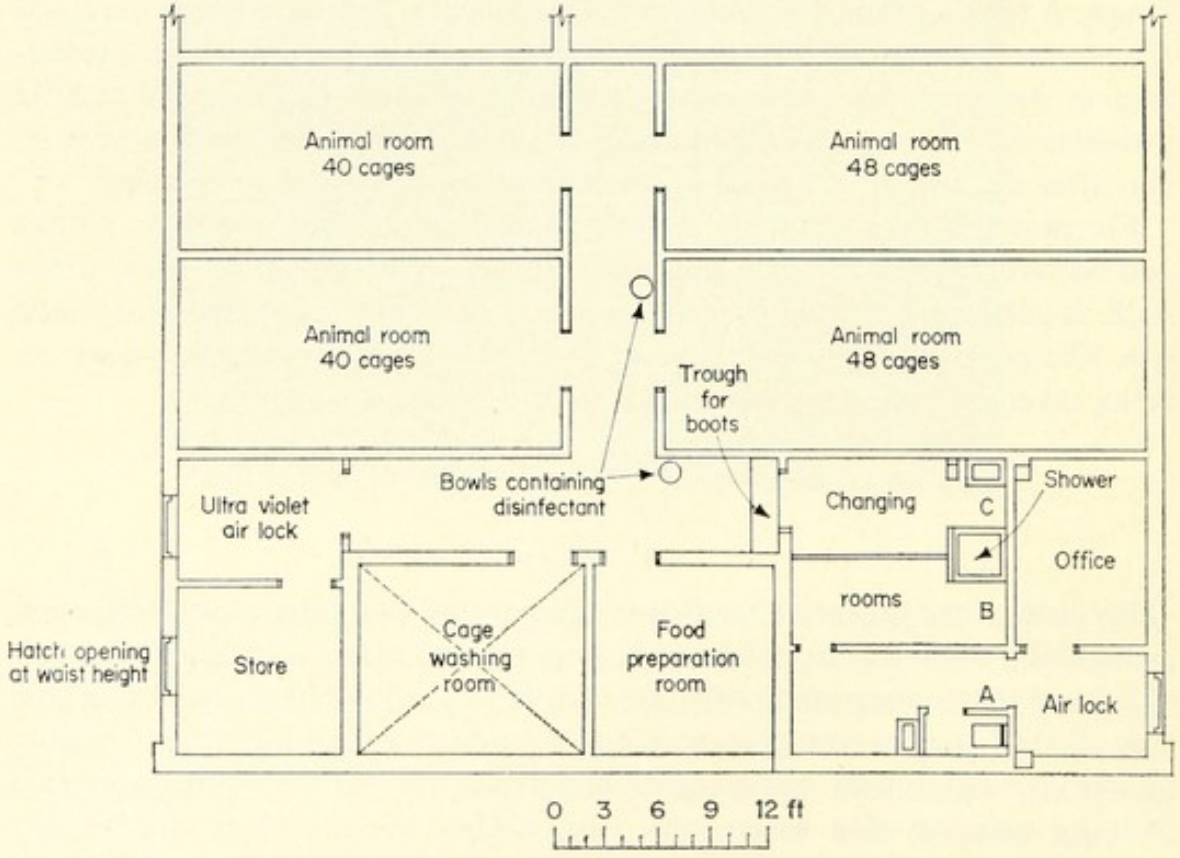

FIG. 3. Plan of animal unit.

hazards to personnel, which have been described previously, there is a greater need for facilities to allow staff to have a complete change of clothing and for bathing. A suitable arrangement for this and other features appropriate for the design of monkey houses has been described by Bywater (1961) and is described here (see Fig. 3).

A. HEATING

Heating arrangement should be such that a constant temperature within a range of 15-27°C (60-80°F) can be maintained. Sudden fluctuations in temperature of more than a few degrees should be avoided and where it is possible to have air conditioning, 10-12 changes per h are satisfactory. Under these circumstances 40 ft^3 (4 m^3) of air space is a reasonable allowance for one monkey. The relative humidity within most animal houses will vary during the day and will be affected by the considerable amount of water normally used for the daily washing routine. A relative humidity in the region of 30-40% with a room temperature of 27°C (80°F) has been found satisfactory (Coid, 1959) and at a temperature of 24°C (75°F) a relative humidity of 45% has been quoted by Wood and Kennard (1956).

B. CAGES

Where large numbers of monkeys are required some institutes keep them in gang cages. These may sometimes contain fifty or more animals, and although this method has the initial advantage in that it is economical to construct, the spread of infectious diseases, aggravated by the insanitary habits of monkeys, is less likely to be controlled than under conditions where they are kept in much smaller numbers. Furthermore, where large numbers are kept together the manipulation, identification and supervision of individual animals is more difficult.

For the majority of laboratories the system which will be found most satisfactory is that where they can be kept one or two in a cage. The layout of suitable cages for this purpose is shown in Fig. 4. Each cage is made of galvanized steel. On the floor of each is a galvanized $1\frac{1}{2}$ in (3·8 cm) mesh underneath which is a droppings tray. At the back, within the cage, is a perch for the monkeys about $2\frac{1}{2}$ in (6 cm) high and $2\frac{1}{2}$ in wide which runs the full width of the cage. Also at the back is a wire mesh grid with external handles fitting into slits running along the sides. By drawing the grid forward the monkeys may be brought forcibly to the front.

The size of the cage shown in the Fig. 4 is $26\frac{1}{2}$ in (67·3 cm) high, $25\frac{1}{2}$ in (64·8 cm) wide and $23\frac{1}{2}$ in (60 cm) deep. These are suitable for keeping monkeys which weigh about 3 kg. Where larger monkeys have to be kept, bigger cages of similar design have been found satisfactory. The size of cage required must necessarily be determined by the size of monkey and the length of time which it is to be kept. A rough guide for the height may be obtained by allow-

ing a few inches more than required for the monkey to stand upright. The minimum depth and width should be two-thirds this dimension.

The droppings tray in each monkey cage should be cleaned daily and fresh sawdust or other suitable absorbent material added. At regular intervals, and always between different batches of monkeys, the cages should be sterilized and scrubbed. Sterilization should preferably be done by autoclaving or with steam at atmospheric pressure. Where it is not possible to use either of these methods, chemical sterilization may be used (Perkins and Short, 1957; Coid, 1959; Bywater, 1961).

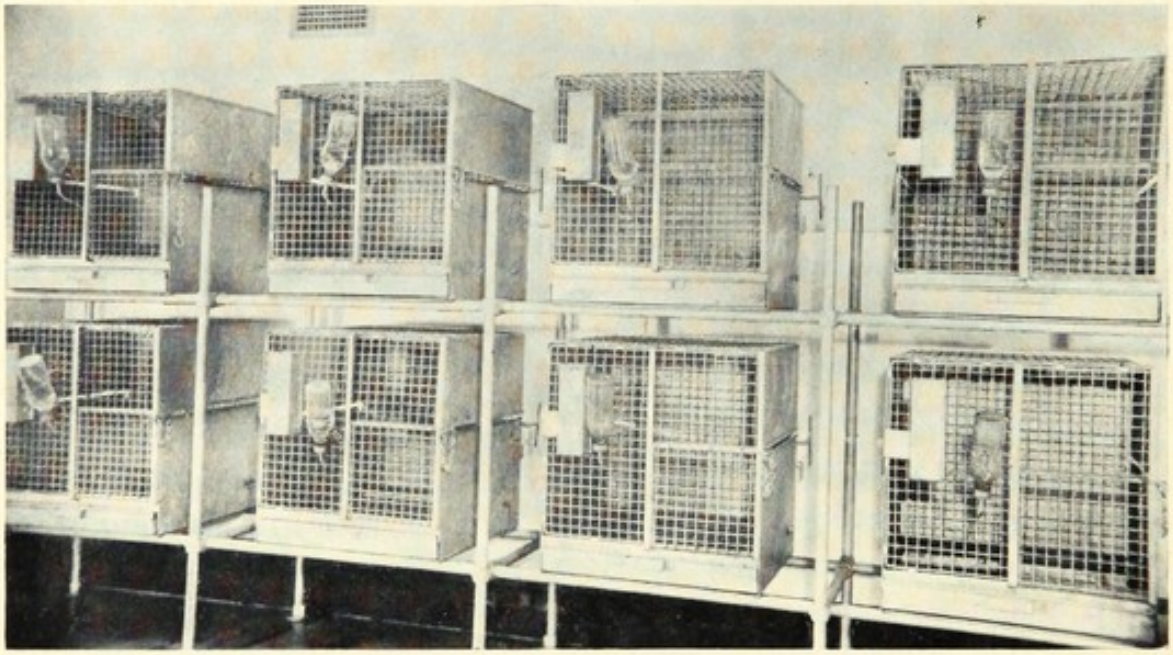

FIG. 4. Layout of cages.

VI. RESTRAINT AND HANDLING

It is likely that the monkeys acquired by many laboratories will have been caught recently from their wild state and will not be used to handling. It is important, therefore, that safe handling techniques are employed and the risk of bites will almost entirely be eliminated if animals are anaesthetized before handling, where this is possible. If an animal has to be caught without an anaesthetic it may be done firstly by manoeuvring it into a suitable position in the cage with the moveable grid so that it is forced to the front and faces away from the door of the cage (Fig. 5). The operator lifts the slide-up door, grabs the tail or leg with one hand and catches the monkey behind the neck. It is then removed from the cage, placed firmly face downwards on the floor and its arms secured behind its back.

Use may be made of a catching box 36 cm × 15 cm × 15 cm which has sliding doors at each end. This is satisfactory for monkeys weighing up to about 3·6 kg. The box is held in front of the cage opening, the monkey induced

to enter it and the sliding door of the box is then replaced (Fig. 6). The catching box is placed on the floor and the monkey is pulled out backwards by the legs and restrained in the same manner as that described after removing it from a cage. Some operators wearing thick leather gauntlet gloves prefer simply to grab a limb while the animal is within its cage. It is then removed and secured by fixing its arms behind its back. This method is unsatisfactory for large animals and even quite small monkeys can bite through leather gloves.

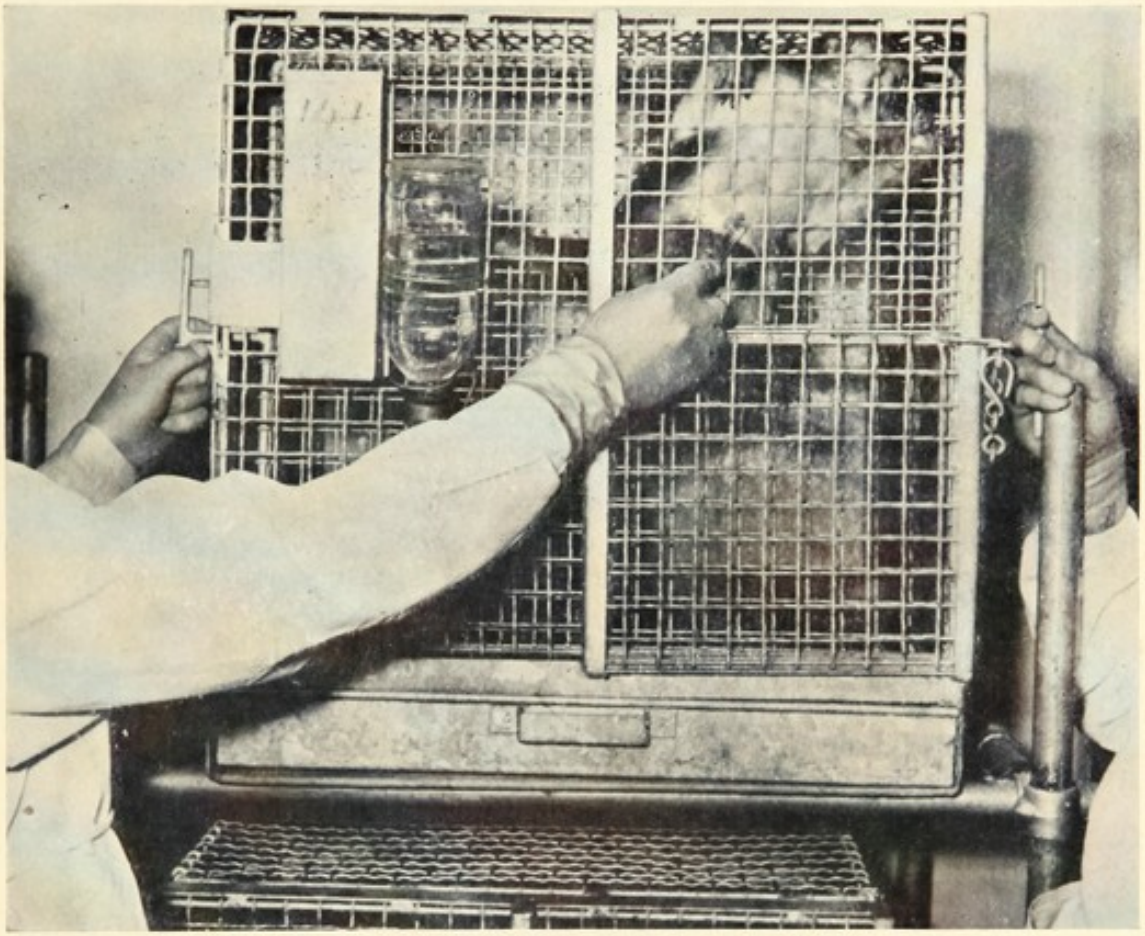

FIG. 5. Intraperitoneal injection through mesh of cage.

A. ANAESTHESIA

The choice of anaesthetic must depend on the procedures to be carried out, but for a large number of manipulations pentobarbitone sodium is useful. A dose of 27 mg/kg body weight may be given intraperitoneally or intravenously. The intraperitoneal administration may be used when monkeys are to be anaesthetized in cages which have a means of forcibly bringing them to the front, and the injection may be given through the openings in the mesh. Intravenous anaesthesia can be given in the saphenous vein which is quite prominent on the posterior aspect of the leg. The animal is held with its

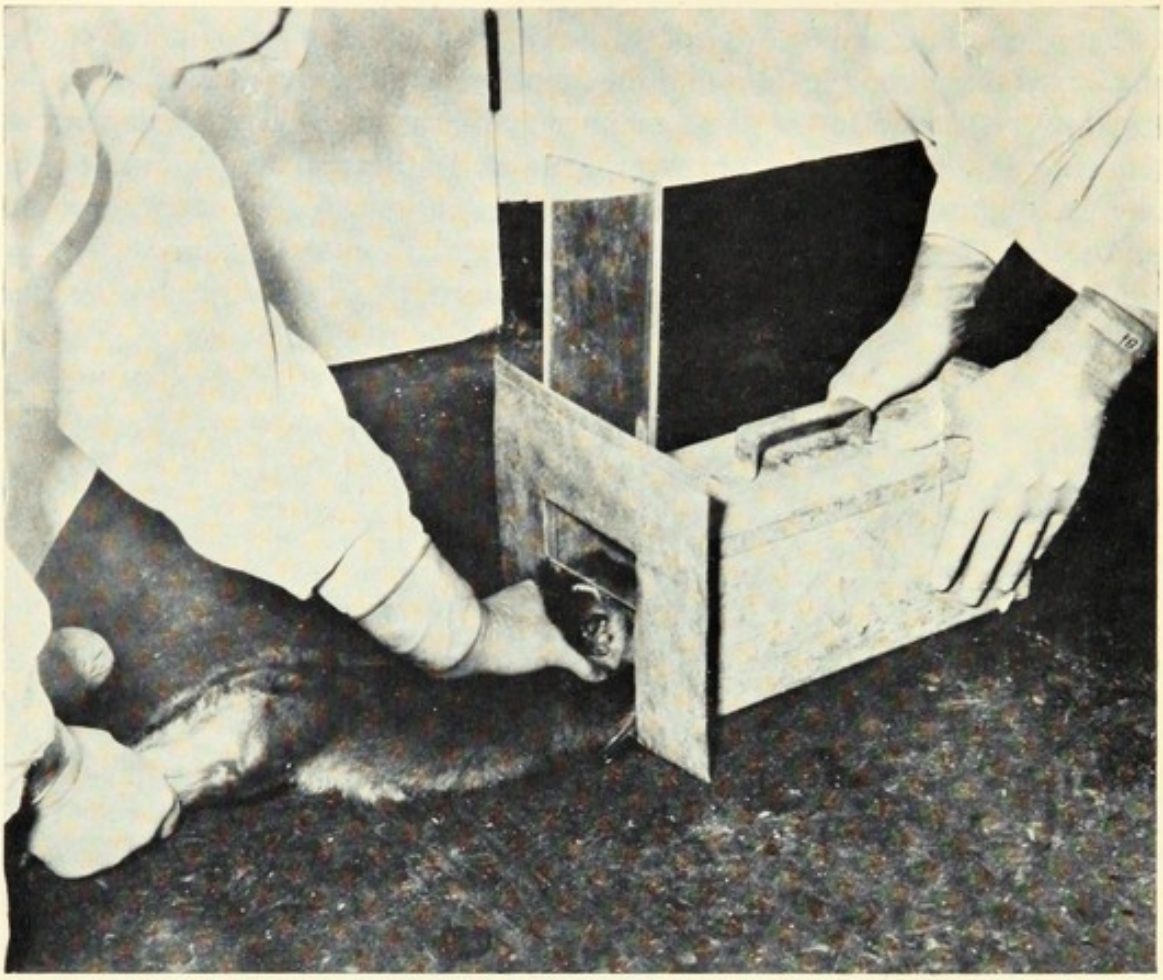

FIG. 6. Removal of monkeys from catching box.

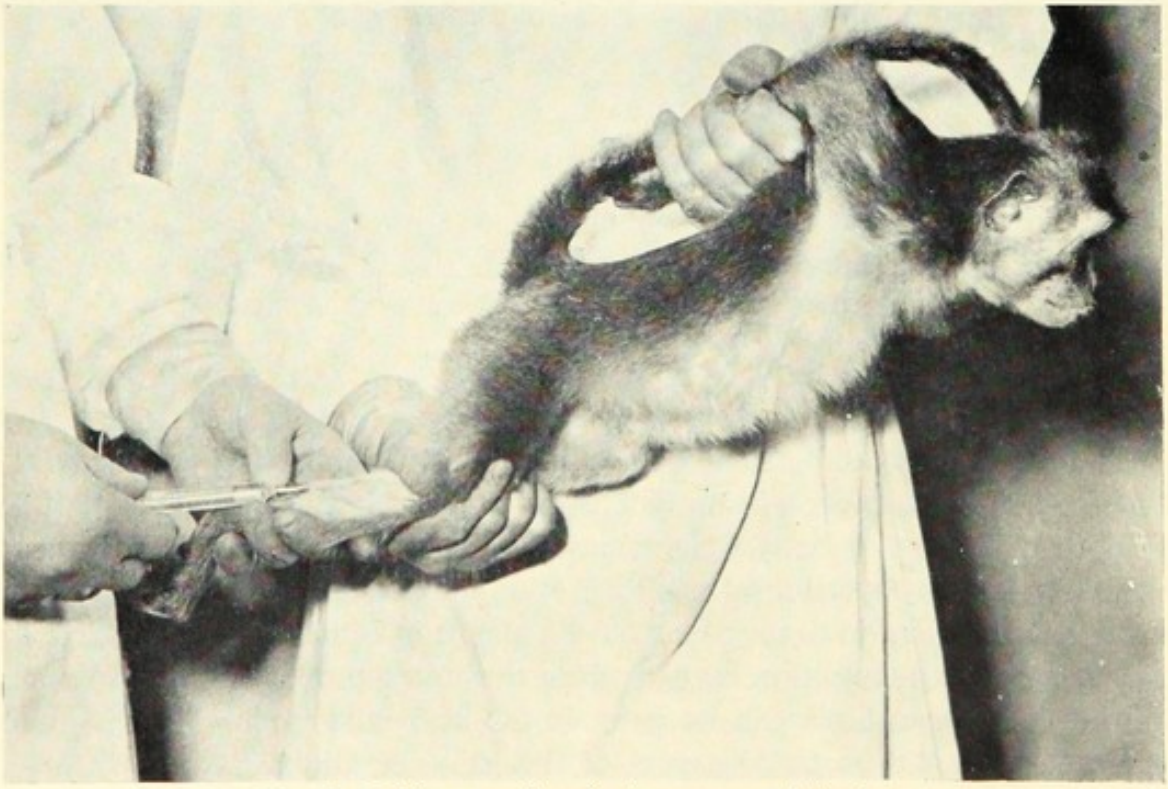

FIG. 7. Holding monkey for intravenous injection.

arms behind its back and the vein is brought into prominence by an assistant applying pressure in the area of the popliteal fossa (Fig. 7). Inhalation anaesthetics may also be administered and these are normally well tolerated. A method suitable for dental and other manipulation procedures lasting several hours consists of rendering the animal unconscious initially with nitrous oxide and oxygen (Cohen, personal communication).

Four per cent Fluothane* (halothane B.P.C.) is then added to this mixture to induce satisfactory relaxation so that an endotracheal tube (Magill, size 00) may be inserted through the nose into the trachea. The anaesthesia is sustained by using nitrous oxide and oxygen and 1% Fluothane.

VII. Feeding

Compounded cereal diets supplemented with additional protein and vitamins are satisfactory for the basic diets of laboratory monkeys. The composition of such a diet, 41B (Bruce, 1958) is given below and, supplemented only with cabbage or fruit daily, is satisfactory for most species of monkeys. Monkeys fed this (about 85 to 140 g daily, depending on size) have bred and been maintained in good health for several years (Short, personal communication).

Diet 41B

	Composition (%)
Wheatmeal	47
Sussex ground oats	40
White fish meal	8
Dried skimmed milk	3
Dried yeast	1
NaCl	1
	100

To each ton of diet is added:

Vitamin A	4,000,000 units
Vitamin D_3	1,000,000 units
Vitamin B_2	1·5 g
Vitamin B_{12}	0·00325 g
Vitamin B_1	0·5 g
Pantothenic acid	0·5 g
Nicotinic acid	2·5 g
Vitamin E	1·25 g
Vitamin K	0·5 g
Choline chloride	25·0 g

and 2-3% of molasses to bind the cubes.

It may be necessary, however, with animals which have recently arrived at the laboratory, to give a small quantity of other suitable food such as mixed grain or wholemeal bread, until they become accustomed to the new diet.

* Imperial Chemical Industries.

Vitamin C is an important constituent of the diet and in addition to supplying food containing this vitamin it may also be added to the drinking water. Newly imported monkeys are frequently found to be suffering from vitamin C deficiency. In addition to compounded cereal diets, it is possible to feed monkeys and keep them in good health on a diet of boiled potatoes, stale bread and vegetables *ad lib* supplemented by vitamins A, B, C and D. When this diet is fed the monkeys should have access to iodized salt (Ekstein and Zuckermann, 1957). Water should be provided *ad lib*. The amount required daily varies with individual animals. It was observed by Wood and Kennard (1956) that at a temperature of 76°F (25°C) and a relative humidity of 45% the average amount of water consumed by monkeys about 5 kg in weight was 0·45 l daily. The water containers should be situated in such a manner that the contents can not be fouled by the animals. A satisfactory method is that whereby inverted bottles, with suitable mouthpiece, are attached to each cage as shown in Fig. 4.

VIII. Identification

Where monkeys have to be kept for long periods tattooing on the lip or abdomen is a satisfactory method of numbering. Small metal discs with the number of the animal may be attached round the neck with a chain. If experiments are to last only for about 14 days a suitable dye, such as carbol fuchsin, can be used for writing the number on the abdomen.

IX. Breeding

Attempts to establish a breeding colony of laboratory monkeys can be frustrating to the uninitiated and experienced alike. Caging a healthy mature female with a healthy mature male in what appears to be a suitable laboratory environment does not necessarily result in conception. Months or years may, and frequently do, pass before pregnancy occurs or it may never occur. Occasionally success from the onset with seemingly few problems is experienced. When breeding does begin, however, it is likely to continue regularly for years.

Breeding stock should be carefully selected from sexually mature and healthy monkeys. Inspection of the sexual skin can be an aid to selection of suitable animals. The sexual skin is that part of the monkeys' body around the external genitalia which swells or shows colour changes during the menstrual cycle. Changes in this skin are not manifested before puberty. The most common laboratory monkey, the rhesus, reaches puberty at about 4 to 5 years. The sexual skin begins to swell and colour immediately after the onset of the menstrual cycle and resumes its quiescent appearance at the end of the cycle. Probably the maximum swelling is reached before the mid-cycle. During lactation the sexual skin becomes inactive.

The menstrual cycle of the rhesus monkey is 28 days and for the other species more commonly found in the laboratory is generally 30 days. The cycle continues the year round. Theoretically, therefore, the monkey can conceive three times a year. Taking the first day of menses as being day 1, ovulation occurs between day 9 and day 18. Probably, therefore, the most successful time for mating will be between the eleventh and thirteenth days. Mating after this time may be too late for conception to occur (Van Wagenen, 1945). Young monkeys are usually more irregular in their cycle than are the more mature females.

Once suitable stock has been selected for breeding, males and females before mating should be caged separately but housed in the same room where both sexes can see and communicate with one another. Caging the monkeys separately is advisable because males, especially, tend to fight when caged together, and disrupt the tranquillity of the monkey quarters; females caged singly are more easily observed and examined for the beginning of menses. It is important that before mating both male and female become acquainted and accustomed to one another. This is especially true of the female; and before being transferred to the male's cage, she should have the opportunity to observe and "talk" to him from the sanctuary of her own cage. Mis-matching of individuals, may well account for much of the failure to establish breeding pairs. It sometimes happens that pairs of primates caged together appear to be living amicably, but upon longer and closer observation are seen to have little affection for one another and under these conditions conception is unlikely. Routine examination of the females daily for evidence of mense will establish the time to transfer the right female to the right male's cage. If females are caged individually, examination of the droppings trays for evidence of blood or discharge before these are cleaned out will facilitate the examination. It is important to make sure, however, that the blood is of vaginal origin and not from some wound or a case of dysentery. By keeping accurate and adequate records, the onset of menses can be anticipated almost to the day in the case of those females having a regular cycle, and a considerable amount of time devoted to inspection can be saved. The best conception rates are obtained if breeding occurs during the mid-cycle. The female should remain with the male for at least 48 h before removing her to her own cage.

The gestation period of the rhesus monkey varies from 159 to 174 days with an average of about 168 days (Coles, 1957). Diagnosis of pregnancy is not easy and frequently not until 3 months have elapsed from the time of mating can it be confirmed with confidence, even with the aid of X-rays. Clinical indications of pregnancy are persistent amenorrhoea, increase in weight, enlargement of the abdomen and colouration of the sexual skin. Rhesus young are usually born at night and weigh probably about 450 g. At birth the infant is covered with thin fur and is usually first discovered

cleaned and dry, clinging firmly to the mother's belly in the morning. The afterbirth is generally not found in the cage; it is usually eaten by the mother. Normally the infant will show signs of hunger within 12-24 h after parturition and will be seen to suck vigorously. By the end of a week the infant's movements will be well co-ordinated, and it will be able to move its head fairly steadily (Dawes *et al.*, 1960). Gradually the young monkey will become more independent and will leave its mother to move about the cage, and to eat food in the cage. It is mainly dependent upon its mother's milk for sustenance, however. The nursing period is probably about 18 months. If it is necessary or desirable, the young monkey can be removed from its mother immediately after parturition. The technique of rearing young monkeys in this way has been described in detail by Van Wagenen (1950).

Breeding monkeys in the laboratory for scientific use is costly, time-consuming and frustrating. Unless there is a specific need to establish a breeding colony, a more economical means of obtaining infant monkeys is the purchase of pregnant females. Fast modern methods of transportation can bring healthy females in the middle stages of pregnancy into the laboratory from their natural habitat in ample time for quarantine and testing before parturition is imminent. As previously mentioned, it is important that satisfactory conditions of transportation are arranged for pregnant females.

Acknowledgements

We wish to express our thanks to Mr. S. A. Keeble for permission to reproduce Figs. 1 and 2 and to Mr. J. E. C. Bywater for permission to reproduce Fig. 3.

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Chapter 16

Domestic Poultry

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I. Introduction

Whilst one might wrangle with the philosophers about the antecedence of the chicken and the egg, one can have little doubt that the startling developments of the commercial poultry industry, in the last 20 years, have presented the research worker with a range of equipment and techniques which must now make the chicken (and perhaps the quail) very attractive experimental animals. Intensive systems of poultry keeping, well-adapted to the needs of the laboratory, are now widely tested and well understood. Requirements for space are comparatively small and the fowl, especially, is very easy to handle. Generation intervals are too long perhaps for many genetic experiments, but the chicken's reproductive efficiency is so high that any studies involving aspects of reproduction in a physiological rather than a genetic sense may be assured of abundant material for their conduct. In particular, embryological studies may be carried through in the absence of the mother. In addition, the importance of the chick embryo in virology and the usefulness of the chick itself for certain bio-assays (e.g. for vitamin D_3) have assured the growing importance of the fowl as laboratory material.

A truly enormous literature has now accumulated on all aspects of the husbandry of domestic poultry. Only a summary of the available information is attempted herein, but reference is made to suitable reviews of the component fields of specialist study.

II. Breeding and Genetics

A. The choice between home-bred and purchased stocks

From a purely technical viewpoint it is not a difficult undertaking to maintain one's own poultry breeding flock and to be able, therefore, to have stocks for experimental work virtually at will. Breeding flocks are nowadays selected and managed to produce fertile eggs all the year round, so that seasonal problems of replacement are no longer serious. This degree of versatility is possible with chicken, ducks and (except for a very short time only) with turkeys.

This has encouraged the development of the large-scale breeder-hatchery man, who has become so important in the modern poultry industry. In the hands of such people—the outcome of whose individual enterprise may well be tens of millions of chicks per year—breeding is enabled to produce material which represents a real departure, genetically speaking, from what was originally possible with the limited numbers within each of the many very small breeding flocks.

The choice, therefore, today is less with questions of the convenience of the laboratory staff, or even of the dangers of importing disease. The really important issue now is the genetic quality and uniformity of the stock that is

to be used, assessed in terms of the likely variability of the measurements that are to be made on the stock. The answer to this question will suggest whether or not it will be worth while to afford the cost of a breeding unit which could give comparable material to that which can now be bought from outside.

B. PURCHASED COMMERCIAL STOCKS

The commercial product is normally a hybrid produced from 2, 3, 4 or more inbred lines. From the point of view of level and uniformity of performance such stocks are very hard to beat. One can safely assume a high degree of selection in the inbred lines and a comparable degree of testing of the matings used to produce the hybrids. To achieve a high degree of testing and selection, large numbers of birds must be used and a high proportion of waste material be accepted. Small breeding flocks are unlikely to be able to produce the premium progeny whose selling price will pay for such a selection programme.

Since many of the measures which one may want to use in poultry experimentation are subject to a high degree of variability (Table I), any improvement in uniformity that can be made is very welcome. The merit of the hybrid as a performer stems, of course, from the high proportion of heterozygous loci that its genotype will possess.

There are now sufficient breeder hatcheries to ensure a reliable supply of stock. It is possible to buy both hatching eggs and day-old chicks. Chicks will normally be available only once per week, but such an interval would be standard even if one were producing eggs from one's own breeding flock and incubating them.

TABLE I

Coefficients of variation for some characteristics of chicken.
(Unpublished data from recent experiments at Reading).

Characteristic	Standard	Coefficient of variation (%)
1. Pullets' age at sexual maturity	Age at 40% rate of lay	9–12
2. Rate of lay	i. 30–40 weeks of lay	17–23
	ii. 10 week periods	26–32
	iii. 3–4 „ „	20–60 (typically 30–40)
3. Egg weight	i. at maturity	12
	ii. at 40, 50, 60 weeks of age	5–6
4. Liveweight	i. 4 weeks (*a*) hybrids	8
	(*b*) cross-breds	15
	ii. 10 weeks—hybrids	10
	iii. 18 weeks—hybrid pullets	10
5. Survival rate	i. Growing pullets	10
	ii. Layers at 6 months	25

C. CONTROL FLOCKS

One problem, however, remains outstanding if one decides to make use of purchased stock, and that is the provision of genetic controls. Though one may purchase only one named hybrid, there is no formal guarantee or even likelihood that it will have similar genetic characteristics from one time of purchase to the next. Consequently, long-term experiments may suffer because one has no knowledge of the new effects of genotype and of genotype × environment interaction that may develop unexpectedly.

There is, therefore, a strong case to be made out for the development of a strain capable of acting as a genetic control. This might be done on a scale appropriate to an individual station or on a national scale. King *et al.* (1959) and Gowe and Johnson (1956) have described the principles and operation of random-bred control flocks. Basically one creates as full a gene-pool as possible and then seeks to perpetuate it against the effects of inbreeding by a programme of matings randomly devised, with restrictions only on the numbers of offspring retained from each mating and on the closeness of subsequent matings. Thus, for example, if fifty males are each given five mates (250 pullets), the matings will be made at random. Initially two sons per sire and two daughters per dam may be saved to rear as the next breeding generation. Subsequently, one son per original sire and one daughter per dam will be used in matings which will be formally random except that matings between half- or full-sibs are excluded.

A breeding flock of this sort could form part of the equipment of any laboratory, on this or even on a somewhat reduced scale. It would go as far as is possible to supply a genetic norm for all types of experiment, allowing the challenge of a constant genetic stock to various environmental backgrounds and providing a common base for selection experiments.

It is true that, from the point of view of management, the structure of a random-bred control flock suggests the use of more males than in a traditional breeding flock, where each sire would have twelve to fifteen mates. But this fact apart, it would be possible to run a control flock in a set of traditional pens. Perhaps, more conveniently, one could make the matings in a range of colony cages.

D. GENERATION INTERVALS

In a control flock the question of generation interval scarcely arises, since no critical time-consuming performance tests are applied before parents are selected for use. In any standard breeding programme, however, the time taken to select parents may materially reduce the rate of movement towards the selection goal, when this progress is expressed per unit of time rather than per generation. If one allows 4 weeks for fertilization of the ovum, oviposition,

incubation and hatching, 20-24 weeks for the growth of the young pullet and 12-16 weeks to secure a minimum part record of yield, then it seems that some 10-12 months represents the minimum theoretical generation interval. In practice, it is more likely to be 18-24 months. If no aspect of adult life is to be measured as part of the selection programme, then the generation interval can be reduced to 6 months. In Japanese quail (*Coturnix*) this period can be reduced to less than 6 weeks.

E. GENETICS

The fowl has been very extensively used in breeding experiments and there is now an enormous literature dealing with the inheritance of both its qualitative and quantitative characteristics. The standard review of these studies is that of Hutt (1949).

III. Housing and Management of Adult Stock

A. CAGES

There is little doubt that the most convenient way of housing laying birds for laboratory purposes is in cages. These may be single cages or groups (blocks of two, three or four cages high by any convenient number in length). Cages may be made from a variety of materials, but the framework is usually of either wood or angled metal and the floor and sides are of either light gauge wire mesh or welded metal strips. The floor should be open enough to allow the passage of the bird's excreta but not so wide that it will not retain her egg. Further it is normally sloped toward the front of the cage so that the egg will roll away out of reach and will be easily available for collection and recording. Many types of multiple cage unit are on the market, many of them large enough to carry mechanical cleaning devices and semi-automatic feeding and watering mechanisms. These models have obvious commercial usefulness but are not necessarily the best for the laboratory.

The size of the basic cage in most units to-day is 18 in (48·8 cm) deep by 15 in (38 cm) wide by 18 in (48·8 cm) high at the front (15 in at the back). This cage was undoubtedly designed with a 6-7 lb bird in view and these dimensions should not therefore be taken as standard. Width has been reduced for smaller birds to 10 in (25·4 cm) and even 8 in (20·3 cm) with no obvious disadvantage, but little adjustment has so far been made in height and depth. For the bird housed singly both could almost certainly be reduced. Another alternative is to put more than one bird in a standard cage which can accommodate two 6 lb birds and three or four light hybrids. Larger cages of say 4 ft × 3 ft (1·219 m × 0·914 m) can house twenty to twenty-five at a time. Such grouping, whilst useful for some studies, does not, of course, allow individual interpretation of food consumption and laying performance. It is likely that birds find necessary movement easiest when the shape of the

floor area of the cage approaches that of a square, especially if the feeding and watering troughs are at the front (access side) of the cage. As far as height is concerned, 16 in (40·6 cm) may represent the minimum, but comparatively small increases of 3-6 in (7·6-15·2 cm) in colony cages have shown that such housing can be quite suitable for breeding stock, allowing adequate head room for the cock to tread the hen. Even in cages of normal dimensions, breeding can be practised if use is made of artificial insemination (Gordon and Phillips, 1951).

The front of each cage should contain a hinged or sliding door, usually kept closed by the attachment of the food hopper, so that birds may be removed easily from the cage. Food hoppers for individual cages represent the most useful experimental layout, but they should be carefully designed to minimize wastage and yet to simplify filling and emptying. A sketch of a suggested design is shown in Fig. 1.

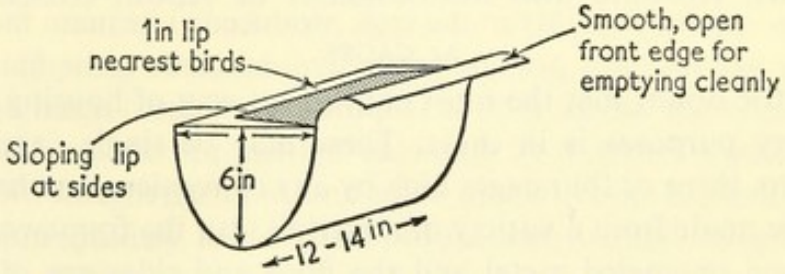

FIG. 1. Suggested design for an individual food hopper.

A drinker running the length of the cage block is most convenient, but for studies involving the measurement of moisture consumption individual drinkers of various designs are available. If the cages are accessible from both back and front, there is much to be said for putting the drinker at the back of the cage. It then stays much cleaner as the bird is not so easily able to plunge a beak full of food straight into the water.

The droppings of the birds should collect on an impervious tray immediately below the birds. The trays can then be removed once every 3 days or even once per week and scraped and washed clean. If the tray is continuous, mechanical scrapers can pull the droppings to one end of the unit at the touch of a button. Mechanical cleaners take scarcely less time than hand-cleaning but they do make the work less of a chore.

B. FLOOR PENS

The only important alternative to cages as housing for laboratory chickens is floor pens. Small pens measuring, say, 10 ft × 8 ft (3·0 m × 2·4 m) or 8 ft × 6 ft (2·4 m × 1·8 m) can be used to house small groups of breeding stock (thirty or fifteen birds respectively). Pens of these dimensions would make viable subdivisions of the animal house and are simple to maintain. The floor

is covered with a litter of sawdust, wood-shavings or peat moss 6-8 in deep (15·2-20·3 cm). This will absorb the droppings of the birds and, if kept dry, will allow almost odourless breakdown of the excreta. Good working litter is essential if birds are not to be exposed to massive doses of coccidial oocysts. Floors of woodens slats or weld-mesh wire may be incorporated in the pen to avoid this danger.

The furnishings of the pen should include roosting perches and nesting boxes. The latter must incorporate a trap in the entrance if individual laying performance is to be recorded. Feeding and drinking troughs can be filled from the access passage in a well-designed set of pens. Nor is it difficult to arrange to empty the nest-boxes from the passage and to return the birds to the pen through a hinged flap in the same wall.

Pens of this sort are particularly suitable for maintaining a breeding flock when each pen produces eggs sired by one cockerel. However, as has been noted, cage-mating is a practicable alternative and may be carried on without loss of fertility or hatchability in the eggs produced. The main fault of the pen system is that it does not provide for individual birds to be handled quickly and quietly, though catching birds is quite easy if the normal lighting of the pen is switched off and very dim blue light is provided on an alternative circuit. In this light birds remain calm and unflustered and there is enough light for the attendant to pick up the birds whilst walking quietly amongst them.

IV. Incubation, Brooding and Rearing

A. SELECTION OF EGGS

Good hatching eggs are those of average weight and shape for the breed of hen. For most breeds of domestic chicken this means a weight in the range $1\frac{7}{8}$-$2\frac{1}{4}$ oz. Eggs of extreme size and shape do not hatch so well and lead to lack of uniformity in the chicks subsequently produced. Egg shells should be smooth, even and free from cracks.

B. STORAGE AND CLEANING

Eggs intended for hatching should be collected every day and racked for storage, broad end up, at a temperature of 50-55°F (10-13°C). This is sufficiently far below the critical value of 75-80°F (24-27°C) to preclude the start of embryonic development. Eggs may be stored in this way for 7 days, but a holding period prior to setting of longer than 10 days will result in a progressive reduction in hatchability. Frequent collection of the eggs will help keep them as clean as is desirable for setting. If it is necessary to set eggs that have been dirtied, they may be cleaned with a buffing pad. It is possible to wash dirty eggs, but this is not recommended for two reasons. The cuticle of the shell may be damaged leading to harmful changes in its

permeability, and infective agents may be transported into the shell pores by the water to such a degree that it would have been better to leave the eggs dirty and rely solely on fumigation for control of disease.

C. INCUBATION

For incubation it is necessary to provide certain controlled conditions of temperature, humidity and ventilation. Almost any container or chamber, from the single egg calorimeters of Romanoff (1941) or Romijn and Lokhorst (1951) to the gigantic walk-in machines of the modern commercial hatchery, can be made to provide these. Commercial manufacturers offer a range of machines varying in capacity from 50 to 50,000 eggs. Air-conditioned boxes can be made on the laboratory bench. It is necessary to provide the following conditions.

i. A constant temperature of 100°F (38°C). Almost any heat source may be used, either inside the incubating chamber itself if even heating can be ensured, or outside, warming the incoming ventilating air. Thermometer readings should be observed twice daily and the constancy of temperature guarded by thermostat and an alarm system.

ii. A relative humidity of 60-65%. This level of humidity will give optimum gaseous exchange through the eggshell consistent with minimum weight loss of the eggs' contents, which should be limited to 9-10% over the period of incubation.

iii. Sufficient ventilation to provide oxygen for, and remove carbon dioxide from, the developing embryos. The gaseous exchange requirements for the individual egg have been tabulated by Romijn (1954) and are given in Table II.

The only other essential characteristic of the incubator is easy access to allow simple loading, testing the eggs prior to hatching and turning them (through an arc of 90-180° according to the type of incubator) at least once a day. Present variation in design of machines would suggest that it does not matter if the egg is predominantly in the horizontal or in the vertical plane, but this is held in question by Kaltofen (1961). In what is likely to be a common laboratory incubator, the so-called table-type, still-air machine, holding 50-500 eggs, the eggs usually lie on their side and are turned by rolling them gently under the attendant's hand.

Testing or candling the eggs may be done at any time during incubation to check for development of the embryo. A convenient time occurs if the design of the machine calls for the transfer of the eggs to a hatching compartment at the 18th day. The eggs are inspected against a background of strong light. A fertile egg shows the shadow of the embryo, an infertile one appears clear and can be rejected forthwith. The number of eggs set minus the number of incubator clears is the normal measurement of fertility in the breeding flock. This should have a value of 85% or better.

TABLE II

Gaseous exchange requirements for the individual egg[1]

Day of incubation	Oxygen requirement (ml/egg/24 h)	CO_2 produced (ml/egg/24 h)
1	—	4·3
2	2·2	5·2
3	2·1	6·4
4	8·5	14·2
5	13·7	15·2
6	22·5	19·4
7	33·3	28·4
8	47·6	36·5
9	64·8	49·4
10	93·1	67·1
11	148·4	99·3
12	239·4	164·0
13	337·0	223·0
14	462·4	301·6
15	483·2	317·6
16	538·0	344·0
17	563·2	381·8
18	556·8	375·8
19	537·6	378·0
20 (after 4 h)	643·6	449·2
20 (after 8 h)	700·0	490·0
21 (hatched chicks)	1436·0	1064·0

[1] Romijn (1954).

D. INCUBATOR HYGIENE

As is widely recognized in culture work, the conditions prevailing in the incubator are also ideal for the development of other organisms which will use the egg as a growth medium. Among these are some which are powerful disease agents in poultry, especially various *Salmonellae*. Fastidious cleanlines of the machine is, therefore, essential and each new loading of eggs should be fumigated with formaldehyde gas produced by mixing 4½ oz (123 g) formalin and 3 oz (85 g) permanganate of potash for each 100 ft³ (2·8 m³) of incubator space (Lancaster and Crabb, 1953). This fumigation should not take place between the 24th and 96th h after setting and is best done before embryonic development has had a chance to start.

E. HATCHING

Hatching is to be regarded as a separate part of the operation calling for extra care. It will be completed, if the eggs are left undisturbed, from the 18th to the 22nd day. On the 19th and 20th days the relative humidity may be reduced to 55% or less (wet bulb 86°F (30°C)) in order to dry out the egg shell and allow maximum gaseous permeability when the chick is commencing to pip the shell. Thereafter humidity is raised again to 70% r.h. or higher so that the early-hatched chicks do not become desiccated. During this period ventilation requirement is at its most critical, and the system may be

calibrated, among other ways, by analysing a CO_2 disappearance curve Romijn *et al.*, 1962).

Provided no pedigree record of the stock is required, chicks may run free on the hatching tray; if chick identity is needed, then eggs of the same parentage should be segregated in perforated metal containers or muslin bags or by the dividing partitions in a specially designed pedigree tray.

The hatchability of eggs is calculated by expressing the number of chicks produced as a percentage either of the fertile eggs picked out at candling (% hatchability of fertiles) or of the total set (% hatchability of all eggs set). The former figure will more quickly show faults in technique of incubation, the latter is the more critical economic figure and may reflect faults in any or all of the following: fertility of either sperm or ovum, nutritional status of the hens, efficiency of disease control and operational technique.

The number of chicks hatched may be increased by continuing the process of incubation for a further 24 h, or by helping out those chicks which are clearly alive but not free of the shell at the time the hatch is taken off. Such practices may improve the hatch by 2 or 3% but are not worth while commercially where the machine is required immediately for a further setting of eggs. Chicks so produced may thrive quite satisfactorily but probably not at the same rate as those hatched more quickly. Some success has been claimed for puncturing the shell over the air sac at the 18th day with a view to speeding up the average time of hatch, but this finding has not so far been substantiated by recent critical work in Holland (Visschedijk, 1962). Visschedijk has shown that 70% of the gaseous exchange prior to pipping takes place through the shell covering the allantois and, further, that the effect of covering or opening the shell over the air cell is to advance or delay pipping but not to affect time of hatching. The variation in hatching time of individual chicks may be a matter of greater experimental concern than has previously been allowed and the subject is at present under examination by, among others, Crittenden and Bohren (1959).

The following routine is carried out at hatching.

i. Removal of weak and unthrifty chicks.

ii. Sexing. This may be done by inspecting the genitalia, either by everting the cloaca or by inserting an illuminated spicule into the vent. In certain breeds and crosses use may be made of the sex-linked genes (barring, and silver-gold) to give auto-sexed chicks, when a difference between the sexes in down pattern or colouring is immediately visible.

iii. Identification. This may be done by punching combinations of holes in the web between the toes or, more conveniently, by wing-banding (fitting a small, sequentially numbered aluminium tab around the wing bone, so that it will remain in position indefinitely).

iv. The removal of chicks to the brooding unit either directly or in specially ventilated and cushioned travelling boxes in which they can safely live for 36 h or more.

F. NATURAL HATCHING USING A BROODY HEN

A broody hen incubates a clutch of eggs with the heat of her own body, sitting on them except when she gets off for food or exercise right through the incubation period. Though it is not now usual to hatch hen eggs this way when artificial incubation is so efficient, a note is included on the method since hens can be used to incubate small numbers of eggs of other species and also because the technique is normal for various small caged laboratory birds such as the canary.

The sitting bird should be provided with a suitable nest in a quiet part of its pen or cage. The nest box should be matched in size to the bird in question (chicken 14 in (35 cm) square, canaries 2-3 in (5·8 cm) square) and should be enclosed except for the entrance and some ventilation holes at the top of the walls. Suitable bedding materials may be clean straw, moss or hair according to the size of the bird. Both birds and nesting materials should be thoroughly dusted with insecticide powder. In the case of birds laying their own setting of eggs, the eggs should be replaced by dummies as they are laid and stored until the clutch is complete. The full sitting can then be put under the bird with some assurance that the eggs will hatch all at the same time.

TABLE III

Incubation characteristics for species other than the hen

Species	Egg weight (oz)	Incubation time (days)	Temperature (°F)	Relative humidity (%)	Clutch number for natural incubation
Turkey	2¾–3¼	28	100 (or slightly over)	65	8–10/hen 15–18/turkey
Ducks		28	100	65	9–10/hen
Muscovy		33–35		(70 for days 1–2)	13/duck
Geese					
Chinese		30	99	70	3–4/hen
Embden		35			10–15/goose
Quail (*Coturnix*)	⅓	16–17	99·5	65 (reduce days 13–16)	

For further discussion of the general principles on incubation the reader should consult *Fertility and Hatchability of Chicken and Turkey Eggs*, Taylor (1949); *The Avian Egg*, Romanoff and Romanoff (1949); and *The Hatchability of Chicken Eggs as Influenced by Environment and Heredity*, Landauer (1961). For discussion of the embryology of the chick and the techniques in

which the embryo can be used, reference should be made to *The Avian Embryo*, Romanoff (1960); *Biochemistry and Morphogenesis*, Needham (1950); *Lillie's Development of the Chick*, Hamilton (1952); the review by Bellairs (1960) and papers by Beveridge and Burnet (1946) and Grau (1960).

G. BROODING AND REARING

Chicks require substantial protection against adverse climatic conditions and it is usual, therefore, to brood them indoors under fairly strict control for at least 3-4 weeks. Thereafter, they may be run outside on clean grass until required for use and it has been traditionally thought that such a method will produce the strongest, healthiest chicken. There is now a good deal of doubt that this need be the case, and when commercial units or laboratories require chickens out of season, then semi-intensive, or even indoor rearing has much to commend it.

H. INTENSIVE BROODING

A brooder house or chamber should be a well insulated structure that can be thoroughly and easily cleaned. Basically it is necessary to provide a source of heat which the very young chick can use to help preserve its body temperature, together with an exercise area into which the chick can wander as it starts to acclimatize to normal room temperatures outside.

I. FLOOR BROODING

If chicks are brooded on the floor, on litter of wheat chaff, chopped straw, wood shavings or peat moss, as is common in many large-scale units, then canopies or hovers containing a heating element can be used to create warm centres (on an otherwise open floor) and from which the chicks can venture out to the limits of the pen as they grow. This is a very simple system but is seldom convenient in experimental work. To meet the latter need, the so-called battery brooder would seem to be a nearly perfect piece of equipment.

J. BATTERY BROODING

This can be thought of as a series of chick houses (together with their runs) piled one on top of the other, three, four or five tiers high. The house is a small compartment containing a thermostatically-controlled heat source, solid on three sides and, on the fourth, shielded from the run by felt curtaining. The run is a caged exercise and feeding area with feeding troughs down the two long sides and a drinker across the end. The whole area has a wire-mesh floor through which the excreta fall on to removable metal, asbestos or plastic

trays, which can easily be taken out for cleaning. Such units are commonly available to carry chicks (at the rate of fifty or 100 per tier) up to the age of 4 weeks. Smaller units may be constructed quite simply in the workshop. Basic dimensions are, height 6-8 in (15-20 cm) and floor area proportional to the number of birds allowing 12 in^2 (75 cm^2) per chick to 2 weeks and 24 in^2 (150 cm^2) per chick from 2-4 weeks of age. Small units can be satisfactorily heated with standard 40W electric light bulbs. Approximately $\frac{1}{4}$-$\frac{1}{3}$ of the area is occupied by the warm chamber, and the sides should be long enough to allow $\frac{3}{4}$-1 in (1·9-2·5 cm) trough feeding space per chick.

K. TEMPERATURE

Initially the warm chamber should supply an air temperature of 95°F (35°C) which is then reduced gradually at a rate of 1°F (0·6C) per day as the chicks become able to withstand lower temperatures. The chicks themselves are the best guide to the efficacy of the heating; if too cold, they will crowd together towards the heat source, if too hot they will move away to the limits of the outside run. It should be possible, except in very cold weather, to do without artificial heating after 3-4 weeks.

L. VENTILATION

Good ventilation is required and the brooder house should have 1 in^2 (6·5 cm^2) of outlet area per chick housed together with 1·5 in^2 (10 cm^2) of inlet area per chick. This relationship of inlet to outlet area is recommended to prevent draughts whilst offering free movement of fresh air.

M. CARRY-ON BROODING AND REARING

Chicks show very rapid liveweight gain in their early days and multiply their birth weight by perhaps ten to twelve times in the course of the first 6 weeks. It is, therefore, necessary to transfer them to new accommodation after 3-4 weeks in the intensive brooders. Similar larger units, with no heat source, may be used, certainly up to 8-10 weeks of age, but thereafter the birds may become too crowded. Such carry-on brooders should have 12-14 in (30-35 cm) of headroom and allow $\frac{1}{2}$-1 ft^2 (450-900 cm^2) per bird. At this stage the birds may be put into the cages they will occupy as adults, but this solution usually seems too expensive and often they are put outside. One modern solution is to plan the brooding and rearing house as one with compartments, as for floor-brooding, big enough to take the group when its members are adults. Instead of running the chicks on a litter floor they are maintained on a wire-mesh floor (initially on, perhaps, one-quarter of its area only) on which they can continue until they are ready for transfer to the laying or breeding house. The false floor, of course, confers one of the big advantages of the battery and carry-on brooders; it keeps the birds away from their droppings.

N. EFFECT OF LIGHT ON GROWING BIRDS

One of the main advantages of intensive rearing systems is the control they give over the bird's reactions to light stimulus. These reactions may be important in relation to some types of work and are summarized here.

1. Light and growth

Light does not promote growth (liveweight gain) except in very young chicks and even these can be trained quickly to feed in the dark. Only those light/dark patterns (such as the normal alternation of day and night) which give a long resting period lead to a growth rate which is less than the maximum possible. Continuous dim light, continuous darkness (after training) or short frequency alternations of light and dark, such as 2 h light and 2 h dark every 4 h, all give high rates of growth. Colour and intensity have no direct importance, but low intensities (and perhaps coloured bulbs producing low intensities) reduce unnecessary activity and are useful under conditions of heavy stocking to prevent birds picking at each other's feathers. Recent work on this subject is well surveyed by Cherry and Barwick (1962).

2. Light and sexual activity

Change in length of the photo-period to which birds are subjected does have a marked effect on the rate at which they mature. As the length of daylight increases birds become more active sexually; conversely as daylight decreases the rate of sexual maturation, or of mature activity, slows down. This effect has been the object of much study in various species of wild birds (see, for example, Farner, 1959) and, until recently, has represented an uncontrolled variable in the keeping of domestic fowl. Pullets will show a variation in maturity at different times of the year of as much as 6 weeks. This range can be extended by artificial patterns of lighting. These quantitative relationships have been discussed by Morris and Fox (1958, 1960). Standard maturity can best be achieved by subjecting the birds to a photoperiod which is constant throughout rearing. The light period may be of any convenient length. In terms of subsequent response to light when the bird is adult a short day (6-8 h) is desirable. If the birds are exposed to laboratory lighting, this should be maintained to give a period of lighting equivalent to the longest light period for which the laboratory will be in use. Alternatively, the birds' accommodation can be blacked-out to give the desired photo-period.

Further information on chicken brooding and rearing may be sought in *Modern Poultry Husbandry*, Robinson (1961) or *Poultry Husbandry*, Jull (1947).

V. FEEDING

The nutrition of domestic poultry has received very intensive study for several decades now. As a result it is possible to buy adequately formulated rations for chicks, growing stock, layers and breeders from a substantial

number of reliable compounders. None the less it remains the case that bought feeds are not necessarily either the best or the cheapest, and the following points are worth attention if it is intended to devise a ration in the laboratory.

A. MINIMUM REQUIREMENTS

It should be accepted that the intensively housed bird is entirely dependent on the ration provided. No advantage has been shown for allowing birds access to grass (Black *et al.*, 1957) or for cutting green fodders to put in their cages, which cannot be matched by appropriate formulation. For example, the deep carotenoid pigmentation of egg yolks from birds with access to pasture can also be obtained by the feeding of appropriate ingredients (e.g., dried grass meal) in the ration.

Bearing this dependence in mind it is possible to set down minimal requirements of amino-acids, minerals and vitamins and to relate them to normal intakes of energy. Fullest tables of requirements are compiled by the National Academy of Sciences—National Research Council, U.S.A. (see Table IV). The same source offers similar but less detailed recommendations for turkeys, ducks, pheasants and quail.

B. PROTEIN REQUIREMENTS

It will be readily noted that the minimum requirements for individual amino-acids do not, when totalled, reach 50% of the value suggested for total protein requirement. This is partly because not all the listed amino-acids have equal availability in common protein supplements. As a result it is sometimes necessary to boost the percentage content of individual amino-acids and, in so doing, to raise the total protein percentage (amounts of readily available amino-acids) considerably. If in the future it is economically feasible to remedy deficiencies by using supplements of synthetic amino-acids it will be possible to reduce present-day standards for protein requirements. Optimum performance, however, is likely to come from protein levels well in excess of the minimum requirements.

C. ENERGY REQUIREMENTS

Once feathered, the chicken has a thermoregulatory system which conserves heat well but is less good at dissipating it. As a result the energy density of its food tends to be an effective regulator of the bird's intake. This in turn suggests that protein, minerals and vitamins should be included in the ration to balance a given number of units of energy (Taylor, 1949). This does not conform with the principles embodied in the recommendations in Table IV indicating amounts of protein, minerals and vitamins per unit weight of the ration. Mineral and vitamin deficiencies are most likely to occur with small-bodied birds which have a reduced digestive capacity and which, because of

TABLE IV

Nutrient requirements of chickens (in percentage or amount per pound of feed)

	Chicks 0–8 weeks	Growers 8-18 weeks	Laying hens	Breeding hens
Total Protein %	20	16	15	15
Arginine	1·2		?	
Lysine	1·0		0·50	
Histidine	0·3		?	
[1]Methionine	0·8		0·53	
Cystine	0·35		0·25	
Tryptophan	0·2		0·15	
[2]Glycine	1·0		?	
[3]Phenylalanine	1·4		?	
Tyrosine	0·7		?	
Isoleucine	0·6		0·5	
Threonine	0·6		0·5	
Valine	0·8		?	
Vitamins				
Vitamin A activity (U.S.P. Units)	1200	1200	2000	2000
Vitamin D (ICU)	90	90	225	225
Vitamin K_1 mg	0·24	?	?	?
Thiamine mg	0·8	?	?	?
Riboflavine mg	1·3	0·8	1·0	1·7
Pantothenic Acid mg	4·2	4·2	2·1	4·2
Niacin mg	12·0	5·0	?	?
Pyridoxine mg	1·3	?	1·3	1·3
Biotin mg	0·04	?	?	?
Choline mg	600	?	?	?
Folacin mg	0·25	?	0·11	0·16
Vitamin B_{12} mg	0·004	?	?	0·002
Minerals				
Calcium %	1·0	1·0	2·25[4]	2·25
Phosphorus %	0·6	0·6	0·6	0·6
Sodium %	0·15	0·15	0·15	0·15
Potassium %	0·2	0·16	?	?
Manganese mg	25	?	?	15
Iodine mg	0·5	0·2	0·2	0·5
Magnesium mg	220	?	?	?
Iron mg	9·0	?	?	?
Copper mg	0·9	?	?	?
Zinc mg	20	?	?	?

[1] Methionine may be replaced by cystine so long as the ration contains not less than 0·45% methionine.
[2] Chicks can synthesize glycine, but not quickly enough to support maximum growth.
[3] Phenylalanine may be replaced by tyrosine so long as the ration contains not less than 0·7% phenylalanine.
[4] Several higher estimates have appeared for the high-producing small-bodied pullets in common use today.

high performance, will almost certainly need to be fed diets with energy contents higher than normal. This basic relationship between energy requirement and appetite is well illustrated by Hill's (1962) figures set out in Table V. It will be noted that variation in the amount of food eaten per day is about 22% of the highest value, whereas variation in calorie consumption is less than 8%.

These figures are especially important when set alongside the N.A.S.-N.R.C. standards for total daily food intake for chicken of different ages (Table VI).

TABLE V

Dietary energy level and food intake in layers

Energy content of diet (cal. m.e./lb)	Food intake lb/dozen eggs	lb/hen day	cal/hen day
1050	5·7	0·31	327
1210	5·2	0·29	351
1350	4·6	0·26	348
1390	4·4	0·25	351
1430	4·2	0·24	352

TABLE VI

Daily food intake per chicken (S.C. White Leghorn) at different ages (weights)

	Body weight (lb)	Total daily food intake (lb)
Growing Pullets	0·5	0·056
	1·0	0·095
	1·5	0·119
	2·0	0·139
	2·5	0·161
	3·0	0·189
Mature birds not in production	4·0	0·156
Mature birds in production	4·0	0·241

Whereas birds will normally seek to satisfy their hunger for calories, light birds of this sort cannot readily overeat and are, therefore, likely to be on a sub-optimal diet unless the energy density of their ration is very high.

D. PHYSICAL CHARACTERISTICS OF THE DIET

One other factor that can markedly affect the food intake of birds is the form in which it is fed—as either a mash or a pellet or a crumb. When a bird is fed pellets or crumbs it can eat faster and will sometimes eat more than when fed mash. This is useful in table bird production but less so in the rearing of layers. Pellets and crumbs are also much easier to store and handle from the attendant's point of view. Mashes should always be very thoroughly mixed—vitamins and minerals being incorporated by means of a pre-mix—and the cereal components should be coarsely rather than finely ground. Food should not be allowed to go stale in front of the birds, who should be allowed to clear their troughs completely every 3 or 4 days. High energy foods containing supplementary fats may go rancid and the food should be discarded if this happens. Fresh, clean water should be available to the birds at all times.

VI. Routine Handling and Management

A. HANDLING

Chicken are docile when handled gently and firmly and should offer the experimenter little trouble. Chicks and stock of a few weeks old only should be caught from above and held so that the palm of the operator's hand encloses the wings and so that the fore-fingers on one side of the body and the thumb on the other can pinion the bird's legs. Once the bird is old enough for its wings to have feathered, it should be held by its wings rather than its legs, for it is liable, if frightened, to flap its wings wildly and is then more difficult to bring under immediate control. The catcher's hand should be slid under one wing with the thumb resting on top of the wing close to its junction with the body. The bird can then be rested against the operator's body whilst it is gripped for examination. In this hold the bird's breast should be supported on the palm of the hand, head facing the arm. The legs are then grasped at the hocks between fingers and thumb, two fingers remaining between the bird's legs. In this way one hand remains completely free for examining the bird. In this position birds may be inspected for detail of feather development, and palpated for both condition of the vent and pubic bones and also for the presence of an egg in the oviduct.

B. CONTROL OF VICES

When confined together at high stocking rates, birds will sometimes show a tendency to peck out each other's feathers, or to peck at the vent of a laying bird, or at some open wound. Sometimes these habits lead to overt cannibalism and considerable losses may ensue. There is now no good reason why such an occurrence should be allowed.

C. DUBBING

Males which have large single type combs may be dubbed to avoid the risk of injury to this organ due to fighting or accident. The operation may be done at all ages, with sharp scissors on a baby chick or with a scalpel or razor (cutting from back to front) on an older bird. The wound should be dressed or cauterized to minimize bleeding. Wattles may be cut back at the same time. It should be remembered, however, that, whilst this operation will reduce the risk of an obvious injury, it precludes studies of comb development. As it is a secondary sex characteristic, the development of the comb does provide a measure of the effect of treatments undertaken using gonadotrophins.

D. DE-BEAKING

Most of the harmful effects of pecking can be avoided if birds are de-beaked This process involves the use of a heated knife blade to cut back and cauterize the points of each mandible. The upper beak may be cut well back, almost

to the nostril, and the lower beak is then trimmed to allow a small projection beyond the new length of the upper. This will prevent the bird from securing a pulling grip on any object but will not interfere with its feeding unless the two beaks are made very uneven in length. Electrically heated machines are available to help perform this operation swiftly and safely.

E. LIGHTING INTENSITY AND ACTIVITY

It has now been generally observed that birds are more flighty and more prone to vice when they are subjected to bright light. If in the normal way light intensity in the animal house can be kept in the range $\frac{1}{2}$-2 foot-candles, little trouble from pecking is likely. Failing control by these methods, some may be achieved by fitting spectacles, as they are called, to the offending bird's upper beak. These are like pince-nez spectacles in shape but opaque, so that the bird cannot see directly forward in the line of its beak. Trouble may arise, however, if the bird catches its spectacles between the wires of the cage or elsewhere, when they are liable to tear off, not without damage to the beak.

F. KILLING

Wherever measurements are required of the dead bird, it may be killed quickly and cleanly by dislocation of the neck. With the bird held by the shanks close to his body, the operator holds the head immediately behind the skull between the first two fingers of the right hand. He then forces his right hand downwards, thus extending the neck, and, at the same time, turns the bird's head backwards over the neck. This movement has the effect of dislocating the neck immediately behind the skull. Birds may also be killed by bleeding and for this operation may be first humanely stunned by electric shock.

VII. Special Techniques

A. ANAESTHESIA

The unique nature of the respiratory system of the bird makes the use of inhalation anaesthesia difficult. Because of the air sacs in the body, it is not easy to regulate dosage concentrations. None the less, general anaesthesia may be maintained with ether from an open jar held over the bird's head. Fussell (1961) reports that "ether in combination with pentobarbitone sodium is safe and provides a wide margin between surgical anaesthesia and respiratory and cardiac failure". As Fussell implies, the preliminary to and, in many cases, the alternative to ether-induced anaesthesia is that of anaesthetics which can be administered intravenously. Chloral hydrate and pentobarbitone sodium are such substances. Chloral hydrate at the rate of 0·2-0·4 g will give complete anaesthesia in a 3-4 lb (1·4-1·8 kg) fowl for 15-60 min. The higher value, however, is close to that suggested as a minimum lethal dose. Pento-

barbitone sodium is a very effective general anaesthetic at the rate of 0·5-0·75 ml in chickens (Warren and Scott, 1935) or 1·1 ml per 5 lb (0·5 ml/kg) body weight in turkeys (Durant and McDougle, 1935). Fussell induces preliminary narcosis with intraperitoneal injections of this drug at the rate of 25 mg per kg body weight.

B. BLOOD SAMPLES

Blood samples are collected normally from the brachial vein where it is exposed at the elbow as the wing is turned back. This technique meets the case when large numbers of birds are to be tested for the presence of pathogens (e.g., *salmonellae*), or the identification of blood group types. Alternatively, sterile blood samples may be taken directly from the heart according to the method described by MacArthur (1950) and Gordon and Horton-Smith (1957).

C. SEPARATION AND COLLECTION OF URINE AND FAECES

Fussell describes a technique for isolating the rectum from the cloaca (after opening up the abdomen) and then exteriorizing it with a cannula at a point on the abdominal wall. Details of the technique and the collection of samples are also fully discussed by Richardson, Watts, Wilkinson and Dixon (1960). A similar technique has been employed by Gordon and Horton-Smith for obtaining sporozoites of coccidia (*Eimeria tenella*) from the bird's caecum.

D. RECORDS

It is pointless to generalize on record-keeping for poultry in a laboratory context. The convenient form of record will depend so much on the type of information sought. Two points, however, are worth a comment in that poultry provide a unique situation. First of all the adult bird needs a daily record (or nearly so) of oviposition. Standard trap-nest cards provide for a mark to be made to represent each egg laid. For the experimenter, however, this is unlikely to provide sufficient information and thought must be given to a form of record in which all the relevant information about the egg, in terms of size and quality and time relationship with other eggs, can be set down. Techniques for doing this sort of recording automatically are at present undergoing development.

A recording system which will show the pedigree and time of hatch of each chick must also be developed. The technique of artificial incubation makes it desirable to assemble into batches eggs of many different pedigrees at one time. Some effects of hatch date (see, for example, the importance of light patterns, p. 446) may be of more consequence than genetic differences and the data must be capable of being handled accordingly.

VIII. Disease

It is not proposed to make any attempt to survey the field of poultry disease. There is a very large literature on this subject and a number of authoritative surveys are available. See, for example, Blount (1947), the British Veterinary Association (1940) and Biester and Schwarte (1959) and, for a very convenient summary, Gordon and Horton-Smith (1957).

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Chapter 17

Amphibia

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I. INTRODUCTION

The importance of the amphibia as laboratory animals has steadily increased in recent years. Adult amphibia play a large role as teaching material and in research on a multitude of anatomical, physiological and endocrinological problems. Because of the generally large size of the individual cells in all tissues, amphibians have also been favourite objects for histological, cytological and ultrastructural studies. The most important contribution, however, has come from the amphibia as providers of eggs and embryos for countless experiments on development and of larvae for the analysis of metamorphosis which transforms the aquatic larva into a terrestrial animal. For a long time their usefulness as materials for genetic studies seemed to be limited. However, the development of a technique for transplanting a nucleus from an embryonic cell into an enucleated frog's egg by Briggs and King (1952) opened a new approach to problems of developmental genetics, which is being used extensively in several laboratories. Furthermore, by painstaking observations and controlled breeding extended over many years, R. R. Humphrey (1959, 1962) has been able to discover a number of new mutations in his large laboratory colony of Mexican axolotls. In recent years, genetic analysis has also progressed in several species of anura (Volpe, 1960).

In earlier years, embryologists had to limit their experiments to the normal breeding season of the most favourable amphibian species. Since Wolf (1929)

first discovered that ovulation and spawning can be induced in the frog during the winter months by pituitary hormones, eggs of many species have become available during the greater part of the year, and the maintenance in the laboratory of adult amphibia throughout the year has become more important. The species which can be maintained most successfully in permanent breeding colonies are the Mexican axolotl, the Spanish newt, and, somewhat less easily, the Japanese newt, among the urodeles, and the clawed frog, *Xenopus*, among the anura.

Detailed descriptions of the species of amphibia and of their life histories can be found in the books by the following authors: Cochran (1961), Klingelhöeffer (1956), Smith (1954, British amphibia), Frommhold (1954, amphibia of Central Europe), Stebbins (1951, 1954, amphibia of Western North America), Bishop (1943, salamanders of North America), Wright and Wright (1949, anura of North America). The excellent photographs in Klingelhöeffer and, especially, in Cochran (many in colour) cover a wide range of species from all parts of the world. A great amount of information on the laboratory care and breeding of amphibia is contained in Hutchinson (1950), Klingelhöeffer (1956), Hamburger (1960) and Rugh (1962).

For their personal communications I am greatly indebted to Drs. J. Bagnara, L. Gallien, J. Gurdon, R. R. Humphrey, J. A. Moore, S. Subtelny, V. C. Twitty and E. P. Volpe.

II. General Remarks on Rearing of Amphibian Embryos and Larvae, on Type of Water or Culture Medium to be Used and on Food

If water from a natural pond, a spring or a well is easily available, it is usually preferable to tap water from the city water system, which may be more or less heavily chlorinated and contain as much as 0·8 parts of copper per million, because of the addition of copper sulphate. The chlorine may be eliminated from tap water by allowing it to stand for two to three days in shallow, open containers. More efficiently, de-chlorination is accomplished by passing the water through charcoal filter beds, or by mixing a small quantity of sodium thiosulphate with the water; an amount of the salt equivalent to the size of a kernel of corn (maize) is sufficient for 60 to 80 l water. The presence of even small amounts of chlorine can be detected by mixing a few ml of a saturated solution of ortho-tolidene (3, 3′-Dimethylbenzidine) with 50-60 ml of water, which will turn a greenish-yellow colour. The copper may be removed by chelation with versene (50 mg/l).

Rugh (1962) points out that, in order to sustain normal development and growth of amphibian embryos, the culture water must contain Na, K and Ca ions in the approximate ratio of 50 : 1 : 1. For salamander embryos and

larvae he recommends a urodele growing solution consisting of spring water (10 l), NaCl (14 g), KCl (0·2 g) and CaCl2 (0·4 g). In his laboratory anuran embryos develop well in plain spring water. At the Princeton Laboratory we have not found it necessary to add any salts to standing tap water or well water to raise urodele embryos and larvae. Hamburger (1960) also recommends plain spring or pond water, or boiled standing tap water.

The optimum conditions of the culture water for the growth of frog tadpoles (*Rana pipiens* and *Rana catesbiana*) were investigated recently by Rose (1960). In addition to de-chlorination and versenation, he found heterotypic conditioning of the water advantageous, which consists of keeping a supply of water in large aquaria containing a variety of aquatic plants, snails, tropical fish, and a complex microfauna and microflora. Every day, one-third of the water is removed for the tadpole cultures and replaced with de-chlorinated and versenated water. Possibly, the foreign species remove generally toxic materials, such as lead ions; or add something to the culture medium which is favourable for the growth of the tadpoles. Rose could demonstrate that, when different numbers of tadpoles are raised in containers of the same size, the average growth rate is inversely proportional to the number of individuals. However, in all containers the growth rates of individual tadpoles vary greatly, some individuals growing as well in crowded containers as in those with single animals. The average growth rate decreases with density because more individuals are stunted in crowded cultures. Stunted individuals removed from their larger partners grow well; when they are left in the same container, they die. The growth-retarding effect passes from one individual to the other through the water; culture water taken from a container with larger tadpoles and used for smaller ones inhibits the latter. The nature of the inhibiting agent given off by the larger animal is not known, except that it is long-lasting at room temperature and rather specific. *Rana catesbiana* inhibits *Rana pipiens*, while salamanders, fish or snails do not. The phenomenon of heterotypic conditioning can be explained by the assumption that the foreign species add something to the water which reduces the intra-specific inhibition of *Rana pipiens*, possibly by binding the inhibiting agent.

Heterotypic conditioning may play an important role in maintaining the optimal growth conditions in a natural pond which may be imitated by keeping adult amphibia and rearing the larvae in large outdoor tanks. In the favourable climate of Tucson, Arizona, Bagnara (1961) is maintaining breeding colonies of the South African clawed frog (*Xenopus laevis*), the Eastern newt (*Diemictylus* or *Triturus viridescens*), the Spanish newt (*Pleurodeles waltlii*) and the Mexican axolotl (*Siredon mexicanum*) in a series of concrete tanks, 180 cm square and 90 cm deep, dug below the surface of the ground. Each tank has its own water supply and a drain in the floor which allows regulation of the water level by a series of stainless-steel standpipes. The temperature of the water is regulated by the running tap water which cools

the tanks in the summer and heats them during the winter. In a colder climate the water could be heated by hot-water pipes embedded in the concrete floor; if greenhouse space is available, similar tanks could be installed indoors. Introduction of material from natural ponds has led to the development of a rich plankton which serves as part of the food of the adult amphibia and is supplemented by the periodic addition of bits of liver.

The same tanks are used for the rearing of larvae which, in the case of *Xenopus* and *Triturus*, swim freely among the adults and escape their predation in sufficient numbers to reach metamorphosis. Larvae of *Pleurodeles* and of the axolotl are kept in small cages made of aluminium screening (ordinary window screen) with a sufficiently broad frame of wood to keep them floating. The young larvae feed entirely on plankton organisms which enter through the screen; older larvae are fed also with small pieces of beef liver or of small earthworms which are dropped into the cages. The large volume of flowing water takes care of the toxic effects of metal ions and waste products.

The types of food recommended as optimal for raising amphibian larvae of various species to metamorphosis and to sexual maturity vary considerably among different laboratories, partly because of actual differences in food requirements of different species, partly because of the local availability of certain live foods and the local traditions that have developed over the years. A summary of the most important current information is given in Table I, and further details may be found in the text, in the description of procedures designed for individual species.

Instructions for the culture of the most useful food organisms are given in the following paragraphs.

Artemia. Freshly hatched nauplius larvae of the brine shrimp, *Artemia salina*, are an ideal food for all small salamander larvae. The dried eggs are obtainable commercially, e.g., from the San Francisco Aquarium Society. An oblong glass tray is filled with a 4% solution of non-iodated sodium chloride. A partition which reaches about 0·5 cm below the surface of the solution is placed about one-quarter of the length of the tray. The eggs are sprinkled on the surface of the solution in the smaller compartment where they will spread out into a single layer. The other end of the tray is turned toward the window or a lamp, and the larvae which begin to hatch within 24 h swim under the partition towards the lighted end, where they can be collected with a pipette. The partition keeps the egg shells at the dark end of the tray. The suspension of larvae is mixed in a centrifuge tube with some tap water and the larvae are sedimented in a hand centrifuge; the clear fluid is pipetted off and replaced with tap water under stirring. The procedure is repeated twice more to remove most of the salt solution. The nauplii remain alive in fresh water for several hours. Daily feeding is recommended.

Daphnia. A number of culture methods have been suggested. At Princeton

TABLE I

Food recommended for amphibian larvae, young animals, and adults

Stage in Life History	Urodeles	Anura
Newly hatched larvae	Nauplius larvae of *Artemia* (brine shrimp), *Paramecia*, small *Daphnia*	Boiled spinach or lettuce, green part of Romaine lettuce; for *Xenopus*: dried nettle or alfalfa powder, dried yeast, egg or liver powder
Older larvae	*Artemia* larvae supplemented with small *Tubifex* or *Enchytraeus* worms, *Daphnia*, adult *Artemia*, freshwater amphipods, *Chironomus* larvae, small amphibian larvae	as above
Newly metamorphosed animals (terrestrial)	Larval and adult *Drosophila melanogaster* (mutants such as vestigial, dumpy, cut, are best at first)	*Drosophila*
Juveniles (terrestrial)	Larger *Drosophila* species (*D. hydei*, *funebris*), house flies, hand-feeding with *Tubifex*	Larger *Drosophila* species, mealworms, small earthworms, crickets
Metamorphosed animals (more or less aquatic) and large, neotenic larvae (e.g., axolotl)	Adult *Artemia*, freshwater amphipods, *Tubifex*, *Enchytraeus*, pieces of earthworms, narrow strips of beef liver (essential to bring axolotls to breeding condition)	For *Xenopus*: *Tubifex*, *Enchytraeus*, small pieces of liver; later: earthworms and beef heart cut into small blocks
Adults (aquatic)	*Tubifex*, pieces of earthworms, narrow strips of beef liver, chopped beef heart (recommended for *Pleurodeles*)	For *Xenopus*: earthworms, beef heart cut into small blocks
Adults (terrestrial)	Larger species of *Drosophila*, house flies, mealworms, crickets	Larger species of *Drosophila*, house flies, mealworms, crickets, grasshoppers and other insects

we have followed Hyman's (1941) lettuce-culture procedure. New cultures are started in 8- to 12-l aquaria with 5-6 cm of water, to which one boiled lettuce leaf is added. After this medium has been standing for two to three days to develop a sufficient number of bacteria as food, it is inoculated with a liberal number of *Daphnia*. When the culture is established and the *Daphnia* have multiplied, the water level is raised at intervals to a total depth of 20 cm. New lettuce leaves are added as the old ones sink and disintegrate. Too much lettuce produces cloudiness of the water which is harmful to the *Daphnia*.

Gordon (1950) prefers a large wooden tub, 180 cm across, with 30 cm of water, in which *Daphnia* can be raised in very large numbers if the water is well aerated with an aquarium pump. As food he adds any one of the following fertilizers: powdered skim milk, dried yeast, bone meal or soybean meal, with an occasional small amount of sugar or molasses and vitamin B tablets. Such a culture has been kept going continuously for 6 years. Smaller wooden tubs, 60 cm across, and discarded bathtubs have also been used with success.

Enchytraeus worms or "white worms" (Gordon, 1950). These small annelids can be cultivated in light soil or humus placed in covered wooden boxes of about 30 × 30 cm, or in enamel pans 30-40 cm across. The soil is kept moist, and small amounts of soft bread dipped in milk, or of oatmeal boiled in milk, are added periodically in deep depressions in the soil and covered with soil. *Enchytraeus* grows best at a temperature of 20-22°C.

Tubificid worms ("*red worms*"). Tubificid worms of several genera (*Tubifex*, *Lumbriculus*, etc.) are usually available from aquarium supply houses and can be kept alive in clean, flat pans in running water which aids in washing out the sewage on which the animals feed normally. For a continuous culture, mature worms are placed in glass bowls with 1·5-2·5 cm of washed sand and 3-5 cm of water. The young *Tubifex* hatch from the cocoons deposited by the hermaphroditic parents during the greater part of the year (about 3-9 per cocoon). A temperature of 22-26°C is optimal. As food, live baker's yeast is pressed into pellets of pea size and buried in the sand (Lehmann, 1941).

III. Shipping

With the development of air transportation, successful shipping of live amphibians over large distances has become a simple procedure. It is particularly advantageous that even fully aquatic amphibians do not require immersion in water, which, on the accidental death of one or more of the animals, becomes easily fouled and may kill the rest. One of the simplest methods consists of packing the animals in small groups in plastic bags into which numerous small holes have been punched for the admission of air. These bags are placed in loosely packed damp moss or excelsior inside any one of a variety of containers, such as a cardboard or plastic box or a tin can perforated with air-holes, or in a basket. A large number of salamanders, for instance, can thus travel from Japan to the Eastern United States in 2 or 3 days at relatively low cost, because of the light weight of the package. Frogs ship well in a wet sack of coarsely meshed cloth inside a perforated cardboard box. Axolotls and other salamanders with gills, as well as amphibian larvae, can be transported in pre-cooled vacuum jugs in ice water.

IV. Stage Series of Normal Development of Amphibian Species

To facilitate the identification of the various stages in the normal development of the embryos of various species of amphibia that are used in experimental work numbered stage series have been prepared by different authors. The classical series is that for *Ambystoma maculatum* (*punctatum*) prepared by the artist, Miss Lisbeth Krause, for Professor R. G. Harrison of Yale University. The original water colours were never published, but photographs may be obtained from the Osborn Zoological Laboratory at Yale. Drawings made from these photographs were published by Hamburger (1960) and Rugh (1962). Series for some other species of urodeles were numbered to match, as far as possible, the Harrison stages. In other series, different numbering systems have been used. A complete list of the published stage series, and of some series available in mimeographed form, is given in the references at the end of this chapter.

Table II

Natural breeding habits of common North American and European urodeles[1]

Species		Breeding season (in northern latitudes)	Approximate number of eggs laid by one female, and mode of laying
Scientific name	Common name		
Ambystoma maculatum	Spotted salamander	March-April	125–250, in firm globular mass of jelly
Ambystoma tigrinum	Tiger salamander	January–March	c. 100, in softer mass of jelly
Ambystoma opacum	Marbled salamander	September–October	50–200, laid singly on land, larvae hatch when site is flooded
Diemictylus (*Triturus*) *viridescens*	Eastern newt	April–June	200–375, fastened singly to leaves
Taricha torosa	California newt	December–May	7–29 per cluster in firm, globular jelly
Eurycea bislineata	Two-lined salamander	April–June	50–60, attached singly to under surface of rocks in streams
Triturus cristatus	Crested newt	April–July	200–300, laid singly on leaves
Triturus vulgaris	Common newt	April–July	to 350, same
Triturus helveticus	Palmate newt	April–July	to 400, same
Triturus alpestris	Alpine newt	April–July	to 400, same
Salamandra salamandra	"Fire salamander"	Spring to fall	up to 70 larvae are born, 25–30 mm long

[1] Data for the former mostly from Rugh (1962), Hamburger (1960), Bishop (1943), Stebbins (1954); for the latter from Klingelhöffer (1956), Frommhold (1954), Smith (1954).

V. Urodeles

A. General

In contrast to the anura, fertilization in salamanders is internal. During spawning, each egg is fertilized as it passes through the cloaca, by spermatozoa stored in a sperm receptacle (spermatheca) located in its dorsal wall. The transfer of sperm to the receptacle is accomplished by means of spermatophores deposited by the male on the bottom of the pond or aquarium. The spermatophore consists of a gelatinous cone-shaped base and a small mass of spermatozoa attached to its tip. The elaborate courtship performance which precedes the deposition of spermatophores stimulates the female to crawl over the spermatophore and to pick up the sperm packet with the cloacal lips. The process by which the spermatozoa migrate into the spermathecal tubules is not known. In the latter, the spermatozoa remain alive and functional for months or a year. As a rule, more than one sperm penetrates into each egg, but only one sperm nucleus with its associated centrosome functions (physiological polyspermy). The supernumerary sperm complexes, which develop normally at first, are inhibited from dividing and begin to degenerate when the first cleavage is in progress.

B. Species most widely used

1. Eastern Newt, Triturus (Diemictylus) viridescens

The Eastern newt has been used extensively in a great variety of investigations, including experiments on maturation and fertilization, morphogenesis and regeneration, on metamorphosis, on heteroploidy and the fine structure of chromosomes as it is revealed by their peculiar lampbrush configuration in the growing oocyte. The adult newt is entirely aquatic and can thus be collected throughout the year. Its life history is complicated by a post-metamorphic terrestrial phase (the red eft stage) lasting from 2 to 3 years, during which the animal wanders far away from its native pond and grows from 25-35 mm at metamorphosis to 60-80 mm. At the end of this phase the eft migrates back to the water, under the influence of a prolactin-like component of the anterior pituitary hormones, and becomes sexually mature. The return to an aquatic existence is connected with a second metamorphosis involving structural changes in the skin, lateral line organs and tongue apparatus, as well as physiological and biochemical changes, e.g., in the mode of nitrogen excretion. The intercalation of the eft stage, during which the animals grow slowly, and the length of the life cycle make it impractical to raise this species to maturity in the laboratory. On the other hand, the presence of two metamorphoses, with the second partially reversing the events of the first, is of great interest for the student of the significance of vertebrate metamorphosis and of biochemical evolution (see Wald, 1952, 1958).

In the laboratory we have found it possible to raise animals for 2 years or more by circumventing the eft stage experimentally. While it is rarely possible to induce a recently metamorphosed animal to return to and to feed in the water, as can be done with European and Japanese newts, the water drive can be released experimentally by implanting 0·5 or 0·25 of an adult newt pituitary gland under the skin of a metamorphosed animal. Even implantation on the day of the first moult is effective in inducing the animal to return to the water permanently within 3-4 days. Moreover, we have found that implantation of a part of a pituitary from an adult into a large premetamorphic larva will actually prevent metamorphosis at least in part, so that the animal remains in the water and retains gills of more or less reduced size.

A more efficient way to prevent metamorphosis is to raise the larvae in a solution of one of the well-known thyroid inhibitors: thiouracil (0·04%), potassium perchlorate (0·05%), or 1-methyl-2-mercapto-imidazole (0·002%). We have raised such permanent larvae for up to 2 years, during which they reached a length of 85 mm, i.e., adult size. However, we have not succeeded in bringing either such larvae or pituitary-implanted animals to sexual maturity.

Fertile eggs may be obtained during the normal breeding season, which lasts from the end of March to June, by placing a freshly collected female into a large finger bowl with several sprigs of *Elodea*. The female will deposit several eggs a day, for a week or longer, by attaching them singly to the *Elodea* leaves, usually bending two or more leaves together with her hindlegs, or folding a single leaf, to make a nest. In the spring, the majority of females carry a supply of spermatozoa in their spermatheca which connects with the cloaca and from which spermatozoa migrate to the cloaca to fertilize each egg as it passes through. In nature, courtship and mating begin in the autumn and are continued throughout the winter and spring. Pairs in amplexus can be seen under a sheet of ice. In the spermatheca the spermatozoa remain functional for several months. The egg-laying period of females in the laboratory may be extended by keeping them in a large, well-planted aquarium with sand on the bottom.

It is more efficient to induce ovulation in the laboratory by implantation of frog pituitaries. This procedure makes it possible to obtain eggs as early as the second half of October, as soon as the ovaries have grown to full size. During the winter, many of the females have not yet mated, and it is necessary to place a male with each female in the spawning bowl. The pituitary-implanted female is very eager to mate (Humphries, 1955); this, together with the ever-present mating drive of the male, makes it unnecessary to implant the latter.

Until they are to be used for the experiments, the newts should be kept in a refrigerator at a temperature from 4-10°C, at which the ovaries remain in good condition for 2-3 months. At room temperature, they begin to deteriorate after about 2 weeks. The animals are removed from the refrigerator

once a week, given *Tubifex* worms, and left at room temperature for a few hours to feed. If new collections of newts are made in December and again in the early spring, normal eggs may be obtained until June.

Our standard procedure consists of placing a pituitary from a female leopard frog in a pocket under the skin of the lower jaw of the female newt, with the female lightly anaesthetized in a solution of chloretone (3 g in 1,000 ml of water). A second gland is given 2 days later, and the female often begins to lay on the 1st to 3rd day following the second implantation. The egg-laying period lasts from 6 to 10 days and may be prolonged in some cases by an additional pituitary implant. The total number of eggs laid by individual females varies greatly, from about 10 to over 100. As the time of the normal breeding season approaches, a single pituitary implant may be sufficient to induce ovulation.

Since the female takes from 3 to 5 min to deposit a single egg, the time of fertilization is known only approximately. Artificial insemination is possible but not recommended for this species, since the number of usable eggs (those located in the posterior portion of the oviducts) is always small.

The outermost layer of the jelly capsule is adhesive and opaque and firmly attached to the inner, clear layers at all points. It cannot be removed in large pieces, as is possible with eggs of European and Japanese newts. When the eggs are freed from the leaves to which they were attached, portions of the opaque layer remain behind and leave one or more transparent windows. For closer inspection of the later embryonic stages, the embryos may be removed from the capsule from the tailbud stage on and left to develop in water.

Newt larvae can be raised from hatching to metamorphosis on nauplii of *Artemia*, if these are given in sufficient quantity. It is easier to switch to *Tubifex* worms when the larvae are half grown. The larvae can be raised in individual watch glasses, or in groups in finger bowls. If sufficient food is given there is little danger of cannibalism. Larger larvae can also be released into *Daphnia* cultures in 8 litre aquaria; they grow faster and to a larger size before metamorphosis under these conditions.

When reduction of the tail fin and of the gills indicates the approach of metamorphosis, a small rock is placed in each container, or a bank of fine sand is built up at one side to give the animals a chance to climb out of the water. The young efts are kept in a terrarium and fed with *Drosophila* which are replenished daily. It is more practical to put an unstoppered culture bottle into the terrarium to furnish a continued supply of flies. The observation that the efts often crawl into the culture bottle and eat both adult flies and larvae suggested the preparation of fly cultures in open glass dishes, where the maggots and eclosing flies are more easily available. For larger efts a larger *Drosophila* species, such as *D. funebris* or *D. hydei*, or house flies can be used. Hand-feeding with *Tubifex* worms is also feasible.

We have tried to keep adult newts during the summer in a large, well-planted tank with slowly running water and to obtain normal spawnings again in the following winter. Following pituitary stimulation the females laid an unusually large number of eggs; however, these eggs were of poor quality as indicated by abnormal or deficient pigmentation, and did not develop normally. It is thus necessary to return the old animals to their pond at the beginning of the summer and to collect fresh breeding stock each fall.

2. California Newt, Taricha torosa

Eggs and embryos of the California newt because of their relatively large size, are favourable material for experiments. They are laid in clusters and provide a large number of embryos from one female all in the same stage of development. From the oviducts of females a large number of eggs can be obtained for hybridization experiments. Twitty and his students (1936-1961a) have made an intensive laboratory study of the development of hybrids between *Taricha torosa* and two other species of California newts, *T. rivularis* and *T. granulosa*. For the past 10 years, Twitty has also conducted a unique experiment in nature, using the secluded Pepperwood Creek on a large ranch in Northern California. About 100,000 hybrid larvae were released into this stream between 1953 and 1958; adult hybrids could be collected from the same site several years later, after long and extensive migrations on land. These and related experiments are contributing very important information on problems of speciation, breeding behaviour, migration, and the sensory basis of homing (Twitty, 1959, 1961b).

Because of the extended breeding season, which can be lengthened further by collection of eggs from different latitudes along the west coast, it is not necessary to use pituitary stimulation. Twitty (1961c) doubts that adults mate and deposit eggs normally under laboratory conditions even during their normal breeding season. He raises the larvae on *Artemia* nauplii to the mid-larval period, from then on on adult brine shrimp whichs are obtainable locally and also are the best food for adults. Newly metamorphosed animals have been raised to sexual maturity on this diet. Although metamorphosed animals feed in the water, provision should be made for them to crawl out, which is done simply by tilting the large finger bowls or baking dishes in which they are reared. Laboratory rearing to the adult stage is not recommended because of the slow growth and the long generation time of this species.

3. Japanese Newt, Triturus pyrrhogaster

The Japanese newt is hardier and more resistant to laboratory conditions than the Eastern American newt. Its life history lacks a prolonged terrestrial stage, and newly metamorphosed animals can often be induced to return to the water after a few days. The adults may be kept in a large aquarium or

tank on a diet of *Tubifex*, earthworms, or beef liver, and will breed again the following year. At Princeton we have had some difficulty in obtaining normal mating in the third year of our colony. To induce spawning during the winter, we have used a similar pituitary-implantation technique as with the American newt; we implant two frog pituitaries at the same time and obtain eggs 3 or 4 days later. A single female may deposit from 30 to 90 eggs over a period of 2 weeks. Hamburger (1960) has good success with two injections of two pituitaries each, given 1 or 2 days apart.

The eggs of *T. pyrrhogaster* are larger than those of *T. viridescens*, and the adhesive and opaque outer layer of the jelly capsule can be pealed off completely, leaving a glass-clear capsule. The methods used for rearing the larvae are identical with those for *T. viridescens*. Japanese investigators have succeeded in rearing this species to sexual maturity and obtained normal offspring from them.

4. European Newts of the genus Triturus

All four species, *cristatus*, *alpestris*, *vulgaris* and *helveticus*, have been used extensively for embryological investigations. In general, it is best to collect fresh breeding stock each spring. However, adults have been kept in the laboratory for several years and have bred each spring. In nature the newts leave the ponds after spawning and take up a terrestrial life until the following spring. In the laboratory they may be kept in the water beyond the breeding season and even for the whole year, or they may leave the water only for a short time and may be adapted again to an aquatic residence by gradual raising of the water level. For successful, continuous culture, Klingelhöeffer (1956) recommends a period of hibernation at low temperatures of 2-5°C.

The larvae are easily raised to metamorphosis. Following transformation the animals may be transferred to a terrarium planted with *Tradescantia*. Healthy animals climb on the stems and leaves and pick up *Enchytraeus* worms from the plants; they should be half-grown in 3-6 months. At this time it is advantageous to adapt them again to water by very gradual raising of the water level. Many newly metamorphosed animals, particularly *Triturus cristatus*, do not show any inclination to leave the water permanently. With these, the terrarium phase can be omitted and the water level raised again immediately. Laboratory-raised *Triturus cristatus* may reach sexual maturity in from 1 to 1·5 years, the smaller species in 0·75 to 1·25 years.

5. Spanish Newt (Pleurodeles waltlii) (Gallien, 1952, 1957, 1962)

The male of this largest European newt is more slender than the female and has heavier arms which, during the breeding season, possess a glandular pad, covered with black, heavily keratinized epidermis, on the upper and lateral sides and dark, thickened epidermis on the inner surface, including the hands and digits. The cloaca is turgid.

Ten adults can be kept in an aquarium 50 × 50 × 25 (height) cm, with slowly running water, and pebbles on the bottom, or in a cement tank 1 m square, without pebbles. Under these conditions the animals are prevented from going on land. Twice a week they are fed finely chopped beef heart which is left in the tank for 8 h, then siphoned off, together with faeces and moults. Males and females are kept in separate tanks, and the temperature is kept between 16 and 20°C.

Spawnings are obtainable from early September to early May; during this time, the males are always in breeding condition. Three pairs are placed in an aquarium 50 × 50 × 25 cm, divided in three by glass partitions. Courtship begins almost immediately. During amplexus the male is situated under the female, with its arms hooked over those of the female. The male deposits spermatophores which are picked up by the female with the cloacal lips. The males are then removed; spawning begins 24-48 h after pairing and lasts for about 2 days. The female attaches packages of about ten eggs each to stones, at intervals of 10-30 min, and lays a total of 400-800 eggs.

The larvae hatch at about 10 days (at 16-20°C) and metamorphose between 50 and 90 days. They are first fed *Paramecia* and very small *Daphnia*, later larger *Daphnia* and hashed *Chironomus* larvae or *Tubifex*, finally whole *Chironomus* larvae. If a small number of larvae is kept in a large volume of slowly running water, they grow to larger size and metamorphose later. At the normal time of metamorphosis the animals measure 55-60 mm, at 4 months about 80 mm. The first spawning can be obtained regularly at an age of 16 months.

In Dr. Gallien's laboratory, some females and males have bred every year for 8-10 years; the oldest female is still fertile at 13 years of age. Adults are probably the best breeders at an age of 2-6 years.

6. Fire Salamander (Salamandra salamandra)

This large, terrestrial species, which may reach a length of 20 cm and more, is widely distributed over Europe, with the exception of the north. Copulation and transfer of the spermatophore may take place either on land or in water. The spermatozoa remain viable in the spermatheca until the following year. Pairing appears to take place during the greater part of the year, except in the cold months, as does the birth of the young. At that time the females visit a spring or clear stream and, with their forepart supported out of water by a rock, give birth to up to 70 larvae. Some larvae may still be enclosed in their membranes, but the great majority are free. Occasionally, the larvae are retained for a longer period, and the birth of fully metamorphosed animals has been observed. It takes the larvae from 3 to 5 months to grow from the 25-30 mm they measure at birth to metamorphosis which takes place at a length of 50-80 mm. Adults have lived in captivity for 25 years; they feed easily on earthworms, slugs and grasshoppers.

7. Spotted Salamander (Ambystoma maculatum)

This species usually does not spawn spontaneously in the laboratory, except under the special conditions mentioned below. Pituitary treatment has not been found successful in inducing ovulation and normal spawning. It is still best to follow the routine established long ago in Professor Ross G. Harrison's laboratory at Yale and to wait for a warm rain to occur during a night of the normal spawning season, which induces the animals to migrate to their spawning ponds. Mating adults can be collected at night and will spawn when brought to the laboratory. Eggs in the one-cell stage can be found in the pond on the morning after a rain. The larvae can be raised easily to metamorphosis but are difficult to rear to maturity in the laboratory.

8. Tiger Salamander (Ambystoma tigrinum)

This larger species adapts itself better to laboratory conditions. Hutchinson (1950) was able to maintain a breeding colony at the Morris Biological Farm of the Wistar Institute for three years. After the animals had spawned in the spring they were transferred to a large outdoor enclosure simulating their natural swamp-wood environment. The enclosure was surrounded by deeply sunken cement walls and covered with wire netting. In the fall the salamanders were collected and moved to a cold room kept at 3-5°C to hibernate. Later on, animals were removed at intervals to a spawning tank at room temperature where the females deposited eggs usually during the third night. In this way the spawning season could be extended into the month of June.

9. Mexican Axolotl (Siredon mexicanum) (Humphrey, 1961a, b)

It is best to keep adult axolotls singly in 4-l containers (small aquaria, glass or plastic fish bowls). The water should be changed after feeding, and the containers cleaned occasionally with a detergent. The animals are fed three times a week with large earthworms or, preferably, beef or lamb liver (not pig) which is cut into thin strips and kept frozen until feeding time. Each animal should be given as many strips as it will take quickly on presentation.

Mature males show a marked enlargement of the lateral margins of the cloaca. Spawnings are obtainable from November to June, and from young animals just reaching maturity (at one year) even in the summer months, if the laboratory temperature is maintained at about 21°C. A male and female are placed together in the early evening in an aquarium 30×45 cm, or a dishpan 37 cm across. A thin layer of fine gravel or coarse sand on the bottom will help to anchor the spermatophores deposited by the male and to keep them upright. The water temperature should be no higher than in the tanks of the colony. The animals should be left undisturbed overnight in the dark; the male may emit from 1 to 25 spermatophores. Ovulation usually occurs only after a successful mating and insemination of the female. Spawning

may begin within 18-30 h after the animals are placed together; in most spawnings from 300 to 600 eggs are laid within 24 h. The percentage of fertile eggs is highly variable. While spawning the female may be placed in an aquarium with clean bottom to which the eggs are attached singly or in small groups. The female should be disturbed as little as possible to avoid interruption of the spawning process, and the eggs removed only at intervals of several hours, unless more frequent removal is necessary for experiments on freshly fertilized eggs. If a female spawns early in the breeding season she may be mated again after 6-8 weeks; young females (1-2 years old) may spawn a third time. It is best to replace the breeding animals at the end of their 3rd year.

Artificial insemination involves sacrificing the male and female. Ovulation can be induced by intramuscular injection of 180-220 I.U. of F.S.H. (e.g., the preparation prepared by the Armour Company for veterinary use). When spawning begins 18-24 h later, the female should be disturbed at intervals to prevent normal expulsion of the eggs which will then accumulate in the caudal portion of the oviducts; eggs that have been in even short contact with water cannot be inseminated. The female is decapitated, the oviducts are exposed, and groups of eggs are transferred to a dry covered watch glass. The content of a sperm duct of the decapitated male is mixed with 10 ml of 10% Ringer's solution, the suspension pipetted over the surface of the eggs and left in contact with the eggs for 20 min. The eggs are then covered with water, and the watch glasses immersed in a large bowl of water after a further 20 or 30 min.

Not more than 50-60 eggs should be placed in 1 litre of water in a shallow bowl. Hatching occurs at about 2 weeks, and feeding begins later when the yolk supply in the intestine is used up. Recently hatched larvae of the brine shrimp (*Artemia salina*) are an ideal first food, but small *Daphnia*, or pieces of small *Tubifex* or *Enchytracus* worms may also be used. Larger larvae are fed *Daphnia* or *Tubifex*, or pieces of earthworms. *Artemia* or *Enchytraeus* alone constitute an insufficient diet as is shown by the appearance of edema and small haemorrhages in the skin. Eventually, hand feeding with narrow strips of beef liver is essential to bring the animals to breeding condition within 1 year. Young larvae should be fed daily; after several months feeding may be reduced to alternate days.

10. Two-lined Salamander (Eurycea bislineata)

This small, slender species of the family *Plethodontidae* (lungless salamanders) is widely distributed over the eastern United States and abundant in small streams and along their margins, where they hide under rocks and in other moist places. The female attaches the eggs to the under surface of rocks in running water; the eggs are creamy white, large, and number from 40 to 60. The larvae spend at least 2 years in the stream before they metamorphose

and can thus be collected at all seasons. They reach a length of 50-65 mm before metamorphosis which was described in great detail by Wilder (1925). Mature females can be easily recognized by the large, white ovaries that are clearly visible through the translucent ventral body wall. In the laboratory, ovulation can be induced during the winter months by implantation of two frog pituitaries given 2 days apart; however, the mortality among embryos raised in finger bowls is high.

VI. Anura

A. General

1. Fertilization

In the anura fertilization is external; the eggs are inseminated by the male, which is in amplexus with the female, as soon as they ooze from the cloaca. Fertilization is strictly monospermic; two or more spermatozoa may be made to enter an egg by using artificial insemination with a high concentration of sperm. Cleavage and later development of polyspermic eggs are always abnormal, because of the independent division of the supernumerary sperm nuclei which is synchronous with that of the diploid zygote nucleus.

Table III

Natural breeding habits of common North American and European anura[1]

Species		Breeding season (in northern latitudes)	Approximate number of eggs laid by one female, and mode of deposition
Scientific name	Common name		
Rana catesbiana	Bullfrog	June–July	10,000 or more, in large film on surface, 60 cm across
Rana clamitans	Green frog	June–August	1000–4000, in film on surface, less than 30 cm across
Rana palustris	Pickerel frog	April–May	2000–3000, in firm globular masses attached to twigs
Rana pipiens	Leopard frog	April–May	3000–5000, in flattened sphere
Rana sylvatica	Woodfrog	March–April	2000–3000, in globular mass
Hyla crucifer	Spring peeper	April–June	800–1000, laid singly among plants
Hyla versicolor	Treefrog	May–July	1000–2000 in small packets of 30–40 eggs
Bufo americanus	American toad	May–June	4000–8000, in two long strings
Bufo Fowleri	Fowler's toad	May–June	Up to 8000, in two long strings
Rana esculenta	Edible frog	May	1600–1900, in clumps
Rana temporaria	Common frog	February–April	1000–4000, in large clumps
Bufo bufo	Common toad	March–April	3000–4000, in two long strings

[1] Data for the former from Rugh (1962), Hamburger (1960), Wright and Wright (1949); for the latter from Klingelhöffer (1956), Frommhold (1954), Smith (1954).

2. Pituitary-induced breeding and artificial insemination

The standard technique for inducing ovulation in the leopard frog, *Rana pipiens*, which is possible from 1 September to 1 May and beyond, was worked out by Rugh (1934). It is described in detail in Rugh (1962, pp. 91-98) and Hamburger (1960, pp. 28-32), and has been slightly modified by other investigators (Volpe, 1961; Subtelny, 1962). The procedure is applicable in principle to other species of *Rana* and also to various species of toads and other anura with some modifications which will be mentioned under the individual species, if important.

At the Institute for Cancer Research in Philadelphia, King and Subtelny (Subtelny, 1962) store the mature frogs at 4°C in carefully cleaned 2-l fish bowls with wire-mesh covers; the sexes are kept separate, with not more than 7 males per bowl, or 5 females, with sufficient water to cover 0·25-0·50 of their bodies. The water is replaced with fresh cold water two to three times a week; feeding is not necessary.

To induce ovulation a female is injected intraperitoneally with fresh frog pituitary glands macerated in Ringer's solution. The dosage varies with the season; three pituitaries from female frogs (which are about twice as potent as male pituitaries) are given during the winter months, decreasing to one female plus one male pituitary by March and April. Each female is placed in a glass jar with a small amount of water and kept at 18-20°C for 48 h during the winter, or for 24-36 h in the spring, before eggs are stripped from the oviducts. The injected females may be returned to the refrigerator and stripped again during the following 3-4 days. Before each stripping the females are kept at room temperature for about one hour.

A sperm suspension is prepared by macerating one pair of testes in about 10 ml of 10% Ringer's (or Holtfreter's) solution in a finger bowl and allowing it to stand for about 10 min, during which the spermatozoa become active. The eggs either are stripped directly into the sperm suspension, or the suspension is pipetted over the eggs that were previously stripped and placed in one or two rows on glass slides. After 10-15 min the sperm suspension is allowed to run off the eggs, and the eggs are covered with spring water. Within 15 min the jelly has swollen, and the eggs can be gently detached from the bottom of the dish, or from the glass slides.

3. Rearing of embryos and larvae to metamorphosis and adult stage

The procedures described in the following paragraphs were worked out primarily for *Rana pipiens* (Volpe, 1961; Subtelny, 1962) but are applicable to many other species of anurans, with the exception of *Xenopus* which will be discussed in the appropriate section below.

Frog embryos can be reared singly in watch glasses, or in groups of 20-40 in finger bowls, up to the feeding stage. Beginning at stage 25 of Shumway (1940) the larvae are fed maximally (i.e., more food is given each time than can be

consumed by the larvae until the next feeding) with the green portions of fresh Romaine lettuce, or with boiled ordinary lettuce or spinach. The bowls are washed, and fresh lettuce is added every two to three days. Larvae grow best when reared separately in finger bowls or small white enamel pans, in spring or pond water.

When the tadpoles reach stage XX to XXI of Taylor and Kollros (1946), with both forelimbs protruding, the water level is reduced and the bowl tilted slightly to leave about one-half of the bottom free of water. No food is given during transformation. From stage XXV on (tail completely resorbed) the young froglets are fed *Drosophila*. Volpe (1961) uses plastic containers, about $35 \times 25 \times 10$ cm, with small air holes, covered with gauze, drilled into the sides. The containers are tilted, and aquarium gravel is added at the higher end. A thriving *Drosophila* culture bottle is placed on the gravel.

One month after metamorphosis the frogs are transferred to larger terraria with vegetation and fed mealworms and small earthworms; they can also be force-fed with liver dipped in a paste of brewer's yeast and codliver oil. After another 2-3 months, the frogs are moved to terraria or larger tanks in the greenhouse. During the summer months, a window is left open in the evening to admit night-flying insects. A light bulb dangling low over the top of the open cage or tank will attract them and bring them within reach of the frogs. Out-of-doors, thin-meshed wire cages can be half-buried in the ground, with a similarly placed light bulb attracting insects at night. During other seasons, the animals are kept in a greenhouse or other suitable room and thrive on a diet of earthworms and crickets.

B. SPECIES MOST WIDELY USED

1. Leopard frog, Rana pipiens

See the preceding section.

2. Other species of Rana

With the technique described for *Rana pipiens*, adapted to differences in the normal breeding breeding season and to requirements for dosage of pituitary glands, Rugh (1962, p. 98) has been successful in inducing breeding in the following species: *Rana catesbiana* (May-August), *R. clamitans* (November-July), *R. palustris* (October-March), *R. septemtrionalis* (November-May), *R. sphenocephala*, Southern leopard frog, probably a subspecies of *Rana pipiens*, from the South Eastern U.S. (all year), *Rana sylvatica* (September-March). The procedure should also be applicable to other, non-American species. In general, larger species seem to require a higher dose of pituitaries than smaller ones. Experiments with mammalian pituitary preparations have been mostly negative.

3. Toads of genus Bufo

With toads, pituitaries from other phyla and extracts of mammalian pituitaries and of pregnancy urine have been found effective in inducing ovulation. However, fresh frog or toad pituitaries are still most reliable. Because the eggs are deposited in long strings, with each egg being inseminated by the male in amplexus as it leaves the cloaca, Rugh (1962, p. 99) finds it advantageous to induce amplexus by injecting the male also with an equivalent dose of pituitaries and allowing the male to fertilize the eggs. Breeding has been induced in *Bufo americanus* from October to April, and in *Bufo Fowleri* from November to April. Volpe (1961) has used both natural and artificial insemination with the Gulf Coast toad, *Bufo valliceps*. This species has a very rapid rate of development; larvae reared individually in small enamel pans complete metamorphosis about 28 days after fertilization, at 25°C (Limbaugh and Volpe, 1957).

4. Tree frogs of the genus Hyla

According to Rugh (1962, p. 99) tree frogs may be caught during or just before entering hibernation. Fertilized eggs have been obtained by pituitary-implantation of both females and males in the following species: *Hyla andersonii* (October-April), *Hyla cinerea* (November-March), *Hyla crucifer* (October-March), *Hyla versicolor* (November-March).

5. The South African clawed frog, Xenopus laevis (Nieuwkoop and Faber, 1956; Gurdon, 1962)

Xenopus is a completely aquatic species in its native South Africa where it occurs by the thousands in silty farm ponds devoid of any higher plants. The normal breeding season extends from September to December. Spawning begins within 8-10 h after the surface temperature of the pond has risen above 21°C and is dropping again. A similar management of the temperature may induce spawning in the laboratory, if the frogs are ready to lay.

It is more reliable to induce spawning by injections of gonadotropic hormones into the dorsal lymph sac. In the Oxford laboratory the male is primed with 50 I.U. of a chorionic gonadotropic hormone 24-48 h before mating, and the female with 30 units 16 h before mating, at a temperature of about 21°C. Final injections of 150 units (male) and 300 units (female) are given about 8 h before the eggs are desired. If the animals are in optimal reproductive condition, the priming doses may be omitted and the final doses reduced to one half. Too high a dose causes the ovulation and laying of immature eggs. Spawning often begins in the early morning and may continue for up to 24 h.

The young larvae hatch after 2-3 days and begin to feed on the 5th day, or soon after. They can be raised to metamorphosis on a suspension of dried nettle powder or alfalfa powder. Egg or liver powder do equally well but

make more frequent changing of water necessary. One l of water per two to four tadpoles is recommended.

During metamorphosis, which is reached in 4-6 weeks, the animals stop feeding and become sluggish and are best transferred to shallow water, although they never leave the water completely. They start feeding again before the completion of metamorphosis, on small *Enchytraeus* or *Tubifex* worms. The growing young frogs are switched to earthworms and, later on, partly or entirely to beef heart cut into small blocks. Adult frogs are fed twice a week. The animals should have plenty of room for swimming, with about 3 litres of water per animal provided. Sexual maturity may be reached in 6 (Nieuwkoop and Faber) to 9 (Gurdon) months, depending in part on the amount of food given. Adults should have about 4 litres of water per individual. Nieuwkoop and Faber recommend slowly running water at about 18°C.

6. Painted toad, Discoglossus pictus

Gallien (1950, see also Gallien and Houillon, 1951) has kept a colony of twelve pairs of this beautifully coloured species which is native to South-West Europe and North-West Africa. Males and females are kept in separate aquaria, 35 × 23 × 25 cm, with water to 3-5 cm. A box made of a wooden frame covered with wire mesh is placed on four feet to one side; an inclined plane allows the animals to leave the water and enter the box which has suitable hiding places provided by hollow bricks or pieces of wood. The toads are fed mealworms (ten per animal per week), offered in the box twice a week.

Spawning may be induced almost at any time of the year by selecting a female that has not spawned for 2-3 months and a male with black callosities on the fingers and a grey chin, and isolating the two in an aquaterrarium with a water level of about 15-18 cm, and some rocks or hollow bricks placed on the bottom. With an electric heating element the temperature of the water is brought to 25-26°C. Spawning takes place within 48-72 h, often at night. The fertilized eggs fall to the bottom from where they are collected.

Development of the eggs is rapid. Hatching takes place at 29 h from fertilization at 25°C (at 43·5 h at 20°C); feeding begins at 94 h at 25°C (at 138 h at 20°C), and metamorphosis takes place at 35-40 days. To the usual food, boiled spinach or lettuce, a piece of spleen is added twice a week and left in the aquarium for 3-4 h. The water is kept clear by adding *Daphnia*.

VII. Diseases and Parasites

Much of the scattered information on diseases and parasites of amphibia was collected recently by H.-H. Reichenbach-Klinke (1961) in his book *Krankheiten der Amphibien*.[1] The following notes are taken primarily from this source, which contains a selected list of references.

[1] To be published in an English translation by the Academic Press, London and New York, in a volume entitled "Principal Diseases of Lower Vertebrates".

Bacteria

Tuberculosis in axolotls and newts is caused by *Mycobacterium piscium* which also attacks fishes; in anurans the infective organism was described as a separate species, *M. ranae*. Early symptoms of the disease are a paling of the skin, followed by the appearance of open sores, general apathy of the animal, and loss of weight. In later stages numerous nodules may be found in the liver, lungs, intestine, kidneys and heart. Amphibia seem to be attacked primarily when their natural resistance has been weakened by improper nutrition, harmful chemicals in the aquarium water, extreme temperatures or insufficient light. Correction of these environmental factors may arrest the disease in early stages; no cure is known for more advanced tuberculosis.

A much more serious disease of anurans, fatal for approximately 50% of infected animals, is red-leg, caused by *Pseudomonas hydrophila* and characterized by haemorrhages in the skin of the belly and legs, and enlargement of the lymph-sacs with fluid. Prompt removal of affected individuals, sterilization of the containers and change to a more diversified diet are recommended.

Protista

Flagellates. Trypanosomes may be present in the blood and intestinal fluid but do not seem to be pathogenic. More harmful are *Dinoflagellates* of the genus *Oodinium*. These pear-shaped organisms, 40-80μ in length, are attached to the epidermis and anchored with root-like processes. They may form a grey film on frog tadpoles, axolotls and adult newts. Treatment with copper sulphate (2 mg/l) or trypaflavine (10 mg/l) is often effective.

Sporozoa. Hemosporidia have been found in red blood cells; a massive infection causes general weakness of the animals. Leeches are suspected as intermediate hosts. *Haplosporidia* (e.g., *Dermocystidium*) form cysts in the skin the size of a pin-head and are harmful when present in large numbers. Infection may take place through small crustaceans.

Ciliates. Tetrahymena pyriformis, when it occurs in the brain and connective tissue of axolotl larvae, may become pathogenic. The disc-shaped *Trichodina* is a skin parasite on frog tadpoles, axolotls, and newts. A bath in trypaflavine 1 : 1000 to 1 : 100, depending of the size of the host, is suggested.

Trematodes

The presence of trematodes in the intestine and lungs is usually harmless, but their occurrence in the cerebro-spinal fluid and (as larval stages) in the muscles may harm the host. Snails and nymphs of *Odonata* are known to function as successive intermediate hosts of the larvae of the adult worms living in amphibia. Frequently, however, amphibia themselves are intermediate hosts of larvae (meta-cercariae) which are found encapsulated in the skin

or muscles, and also in the eyes, brain, heart and liver. Snails are the first hosts, and the final hosts, in which the trematodes mature, may be larger frogs, snakes, storks, herons and polecats or other carnivorous mammals.

Nematodes

Infection with nematodes seems to occur through the bite of mosquitoes and is usually harmless, as long as the worms are confined to the intestine and lungs. They may become dangerous when present in large numbers in muscles and blood (e.g., *Filariae*).

Crustacea

The most common parasitic crustaceans are *Argulus foliaceus*, the carp-louse, on frog tadpoles, and, in North America, *A. americanus* on tadpoles and salamanders. In rare instances, the highly modified copepod, *Lernaea cyprinacea*, normally parasitic on fish, has been found on tadpoles of the bullfrog.

Insect larvae

The only known species of insects parasitizing anurans, primarily toads, is *Bufolucilia bufonivora*, a fly which deposits its eggs into the nostrils of the host. The maggots tunnel into the mucous membranes and the bone and may enter the brain. The infection is usually fatal.

Fungi

Infection of the skin with fungi is the most common hazard in colonies of both larval and adult amphibia; as a rule, it is dangerous only when wounds are present, although it is possible that small skin lesions are the usual site of the start of an infection; the hyphae may penetrate the tissues down to the bone. *Basidiobolus ranarum* is found on frogs; brushing of the infected parts with a solution of potassium permanganate (1 g/100 l) or a 10% solution of tincture of iodine may clear up the infection. *Saprolegnia parasitica* has been described from frogs and newts, and *S. ferax* from newts. Disinfection with solutions of trypaflavine or methylene-blue is recommended. Descriptions of the species of fungi involved are given by Tiffney (1939) and Warthmüller *et al.* (1932).

A peculiar and usually fatal disease is described by German authors as "Molchpest". Early symptoms are general apathy, refusal of food, and disturbances of equilibrium, followed by the appearance of reddish patches and open sores, 1-2 mm in diameter, in the skin which eventually may cover the whole animal. The water of the aquarium has a typical parsley-like odour. A secondary infection with fungus is the rule. The primary infectious agent, if any, is unknown. It is suspected that a disturbance of metabolism may be involved, caused by unfavourable environmental conditions.

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