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BREEDING AND CARE OF LABORATORY ANIMALS

VOLUME II

Prepared for the World Health Organization,
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VOLUME II

Contents

	<u>Page</u>
Chapter:	
1. Handling	4
2. Sexing	8
3. Identification and marking	11
4. Recording of data	18
5. Breeding	33
6. Feeding requirements of various species	49
7. Litter	52
8. Sterilization and disinfection: premises, equipment and supplies	55
9. Routine activities in the animal unit	59
10. Mating systems for the maintenance of consanguineous strains and the production of consanguineous animals	64
11. Mating systems for the production of non-consanguineous strains of animals	68
12. Euthanasia	71

BREEDING AND CARE OF LABORATORY ANIMALS

The purpose of this manual, which is intended for developing countries, is to provide basic knowledge about the breeding and care of mice, rats, guinea-pigs and rabbits used for laboratory diagnosis and for the production and control of vaccines, reagents and drugs.

The stress is on the practical aspects. Priority is given to simple procedures that can be applied without extensive resources, for the production of conventional animals of higher quality than the animals generally available in the countries concerned.

More advanced techniques such as the production of "specific-pathogen-free" (SPF) animals are treated as ideas to be considered for the future. They can reasonably be considered only during a second stage when personnel already qualified in the basic techniques are available, together with specific technological support.

VOLUME I is primarily intended for people in charge of the planning, organization, management and operation of the laboratory animal unit, particularly the supervision of the animal keepers. It indicates how to select the techniques and methods best suited for the current local conditions.

VOLUME II is intended, but not exclusively, for the various categories of staff responsible for looking after the animals, and deals with the practical problems they encounter in their everyday work. It concentrates on the problems of breeding and care and does not deal with the procedures involved in the experimental use of animals: e.g., different types of sampling, administration routes for various substances, anaesthesia.

CHAPTER 1. HANDLING

Animal handling is an important matter. Proper handling should ensure:

- the least possible stress for the animal and avoidance of all risk of cuts, bruises, etc;
- the safety of the technician (avoidance of scratches and bites);
- establishment of the indispensable good rapport between man and animal.

The correct handling technique for each species cannot be taught in a theoretical way, but must be acquired with regular practice.

1. General principles

- The animal should be approached gently and unhurriedly, with neither sudden movement nor hesitation.
- Once the animal is grasped it should be held firmly, but not so tightly as to make it struggle or break loose.
- The use of tongs is not at all advisable, except with infected animals which could infect the handler by biting or scratching.
- Pregnant females should be handled with care and only if absolutely necessary.

The handling technique should be adapted to suit the morphology and natural behaviour of each species.

2. Mice

The mouse is the only laboratory animal which can be handled by grasping the base of the tail between thumb and index finger. Never lift a mouse by the tip of the tail since it can turn and bite the handler (Fig. 1).

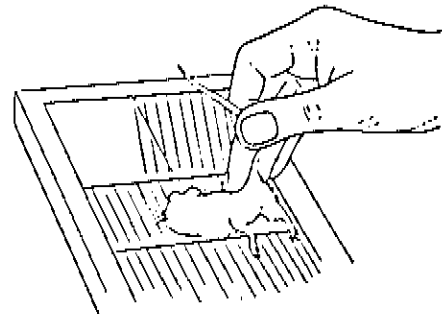


Fig. 1

To immobilize a mouse, hold the scruff of its neck between index finger and thumb, turn the hand so that the animal's back rests along the palm and grasp the base of the tail between ring finger and little finger (Fig. 2).

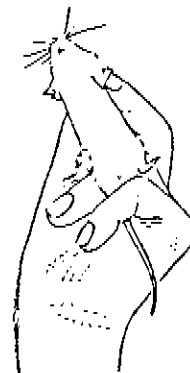


Fig. 2

3. Rat

The rat should never be taken by the tail. Put the palm of the hand on the animal's back and immobilize its head with the thumb (Figs 3 and 4).



Fig. 3

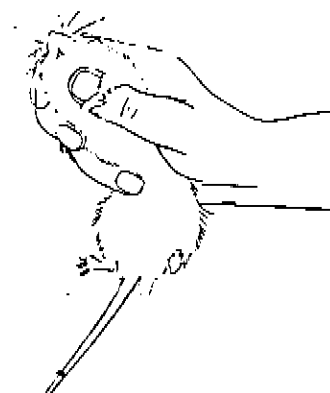


Fig. 4

For more secure immobilization and when dealing with larger animals, both hands may be used (Fig. 5).



Fig. 5

4. Guinea-pig

Same technique as for the rat (Fig. 7). When dealing with adult animals and pregnant females in particular, the free hand should be cupped under the animal's hind quarters in order to support the body weight (Fig. 6).



Fig. 6

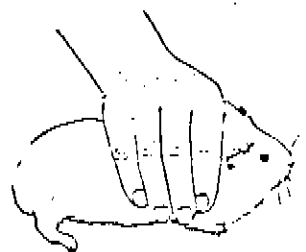


Fig. 7

5. Rabbit

To take a rabbit from a cage, grasp its ears with one hand and place the other hand under the animal's abdomen. Lift with both hands at once.

In order to carry a rabbit, place the index finger between the ears and the other fingers round the animal's head to hold it still. The other hand bears the animal's weight. It should be carried either in a vertical position with its belly against the handler or in a horizontal position (Figs 8 and 9).



Fig. 8

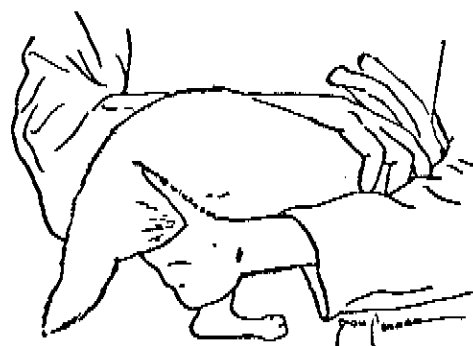


Fig. 9

CHAPTER 2. SEXING

Sexing is a routine operation in the breeding process, since it is necessary to:

- determine the sex of animals in the pre-pubertal period;
- mate the breeding animals whose sex is known.

Sexing of adult animals presents few problems;

sexing of pre-pubertal animals is more difficult since in some species (such as the rat) the testicles do not drop into the scrotal cavity until puberty.

1. Rats and mice

In neonates and young, identification of sex is based on:

- examination of the ano-genital area. The distance between anus and genital papilla in the male is approximately twice that in the female (Fig. 1).

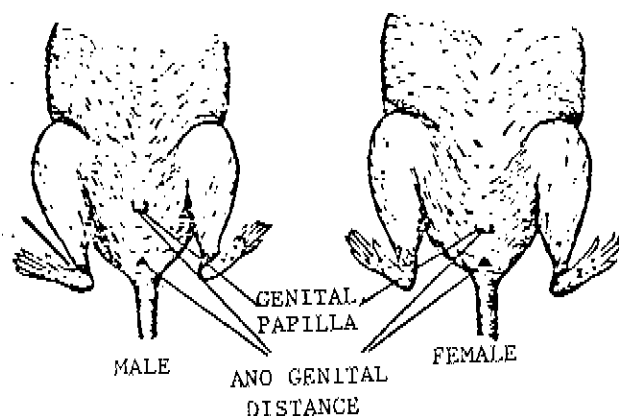


FIG. 1. EXTERNAL GENITALIA OF YOUNG RATS.

Example: rat - average ano-genital distance, measured in millimetres:

	<u>Male</u>	<u>Female</u>
Birth	2.8	1.2
1 week	5.2	2.7
2 weeks	8.2	4.9
3 weeks	12	7

- teats are visible:
 - from third to thirteenth day in female mouse;
 - from eighth to fifteenth day in female rat.

From the thirteenth day in the mouse and the fifteenth day in the rat, the teats are hidden by hair.

- In pigmented strains there are two small, lightly-pigmented patches in the male on either side of the genital papilla. In adults the genital organs are easily identified (Fig. 2).

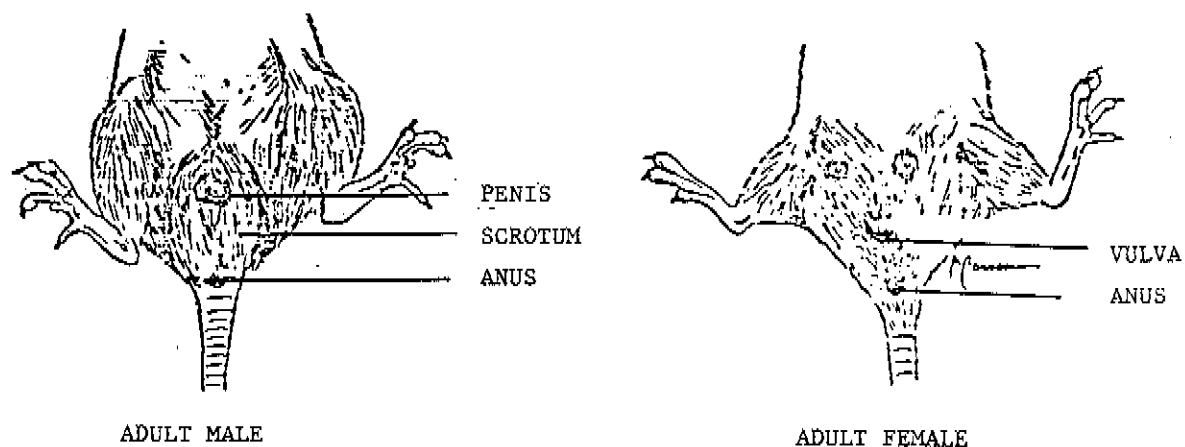


FIG. 2. EXTERNAL GENITALIA OF ADULT RATS

2. Guinea-pig

Anus and genital area are very close together both in male and in female (Fig. 3). The distance between them cannot serve as a criterion. For sexing, the guinea-pig must be held in the vertical position, its back against the handler, whose upper hand should hold the animal by the neck while the lower hand holds the animal's hindquarters, exerting slight pressure at the genital area with the thumb in order to evaginate the penis or display the vaginal orifice.

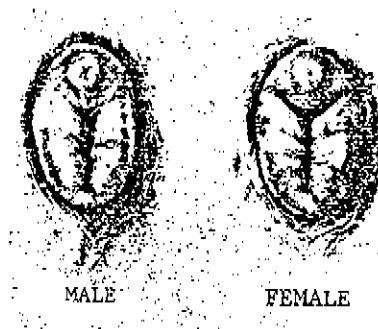


FIG 3. EXTERNAL GENITALIA OF THE GUINEA-PIG

3. Rabbit

The sexing of rabbits under 6 weeks old is a difficult procedure.

Hold the ears with one hand to keep the animal still. With the fingers of the free hand exert slight pressure above the ano-genital region.

- In the male: the rounded tip of the penis appears (Fig. 4).
- In the female: the vagina appears as a fissure stretching towards the anus (Fig. 4)

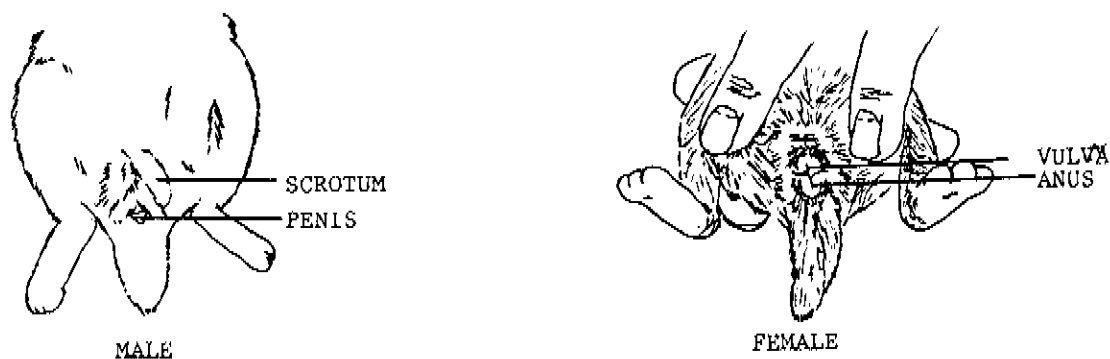


FIG. 4. EXTERNAL GENITALIA OF ADULT RABBIT

For very young rabbits, hold by the hind limbs (Fig. 5) and press lightly with both thumbs on either side of the ano-genital region.

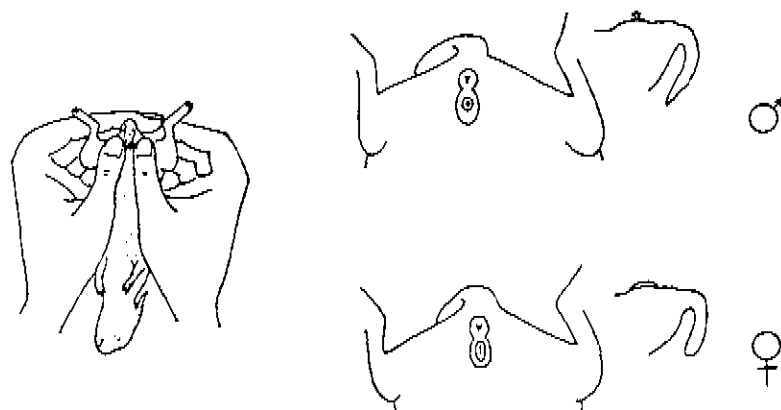


FIG. 5. SEXING TECHNIQUE FOR USE ON YOUNG RABBITS

CHAPTER 3. IDENTIFICATION AND MARKING

It is often necessary or useful to be able to identify animals:

- either individually
 - to ascertain their pedigree (for the maintenance of consanguineous strains);
 - to obtain information on individual performance.
- or in groups
 - to distinguish one batch from another.

Marking procedures are used for animal identification:

- systems for direct reading, consisting of numbers, letters or a combination of the two;
- codes, with marks denoting:
 - a number;
 - a characteristic;
 - membership of a group;
 - performance.

1. Methods

1.1 Identification by code

1.1.1 Colours: laboratory stains are generally used in 3-5% solution in 70% alcohol, with the exception of picric acid which should be prepared in saturated solution:

- blue: trypan blue
- violet: gentian violet
- green: brilliant green
- red: fuchsine
- yellow: picric acid

Technique appropriate for light-coloured animals only:

- the stain is applied with a brush, against the lie of the fur, the length of the animal's coat;
- repeat every three weeks. This marking system is temporary.

The code consists of applying stain to a given location: head, back, base of the tail, right haunch, right shoulder, left haunch or left shoulder. Combinations of two are also possible: head and left haunch, back and right shoulder. (Fig. 1).

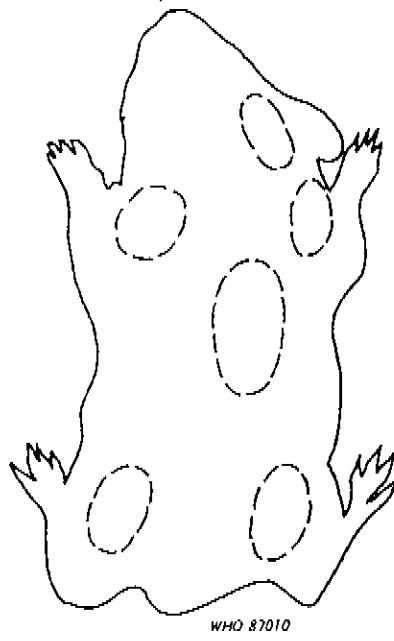


FIG. 1

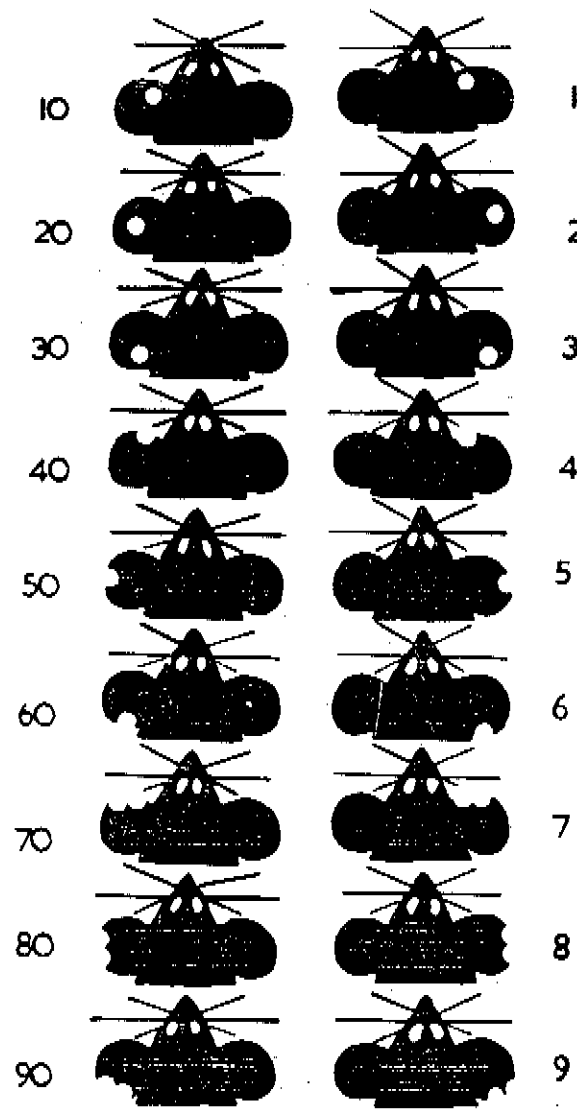


FIG. 2. HOLES AND NICKS ARE USED TO INDICATE TENS ON THE LEFT EAR AND UNITS ON THE RIGHT. THIS CODE GOES UP TO THE NUMBER 99 AND IS USED MAINLY FOR MICE AND RATS.

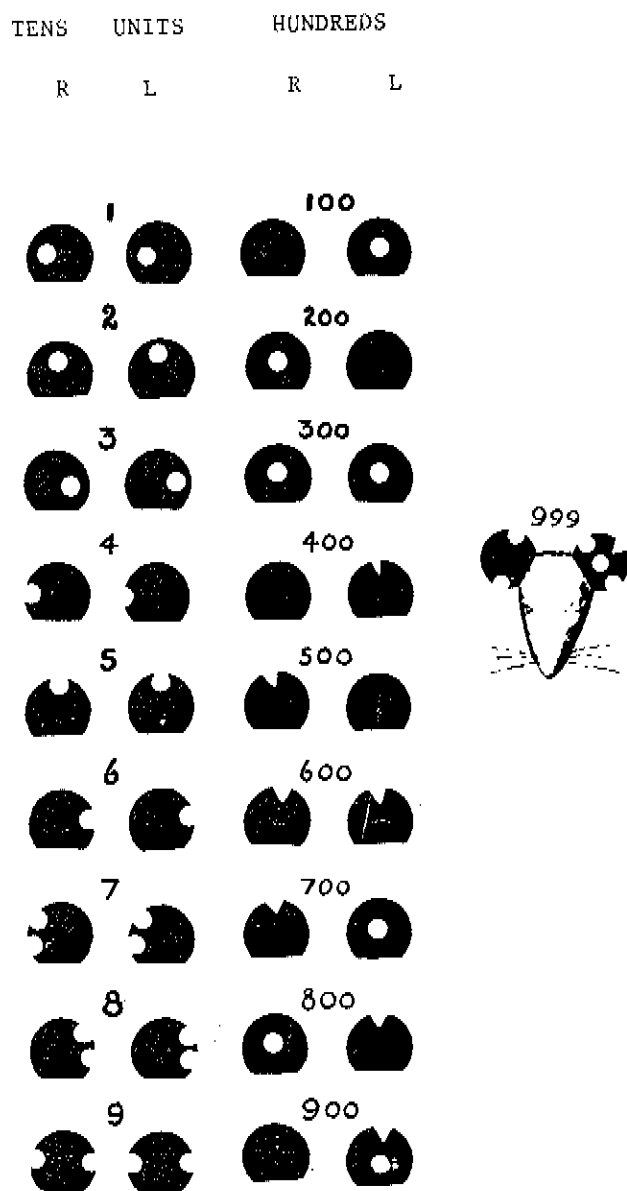


FIG. 3. HOLES AND NICKS ARE USED TO MARK HUNDREDS ON THE RIGHT AND/OR LEFT EAR. TENS ARE MARKED ON THE RIGHT EAR, UNITS ON THE LEFT. THIS CODE GOES UP TO 999 AND IS USED MAINLY FOR RATS.

1.1.2 Ear punching: nicks and holes are punched in the ears, in various combinations corresponding to numbers in a code. There are various codes (Figs 2 and 3). This marking system is permanent.

- Rats and mice: holes 1.5 mm in diameter made with a punch for marking the web of chickens' feet (Fig. 4).
- Guinea-pig: holes 3 mm in diameter made with a leather punch.

PUNCH FOR MARKING RATS AND MICE.



FIG. 4

- Rabbit: notches made in the ears with scissors. This can only be done to newborn animals. For numbers greater than 10 both ears are used (Fig. 5).

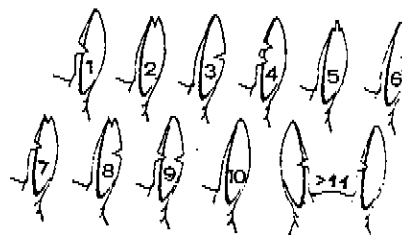


FIG. 5

1.2 Direct identification

1.2.1 Descriptive method: animals with variegated coats are identified by their colour markings:

- by a description of the location and colour of the patches;
- by transcription on to a drawing of the right and left profiles of the animal (Fig. 6);
- by photographs.

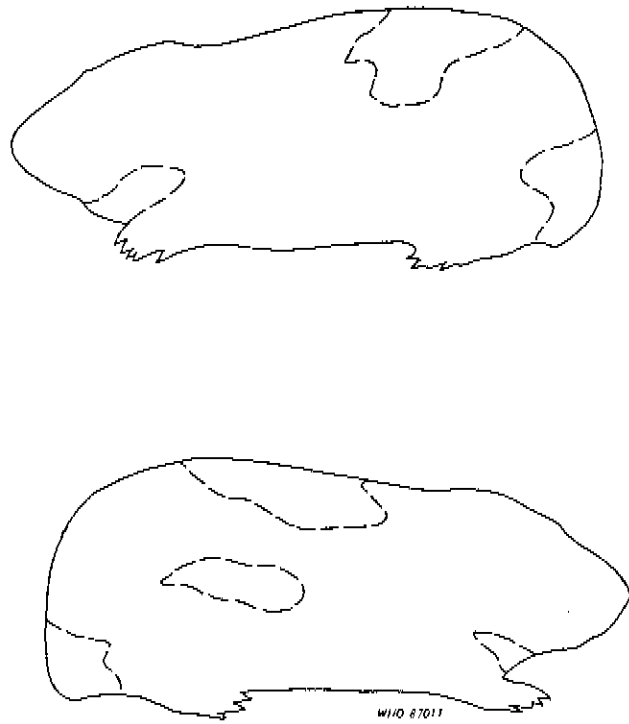


FIG. 6

- 1.2.2 Ear-tags: ear-tags of metal or plastic are stapled on. These identify the animal in clear with numbers or letters (Fig. 7).

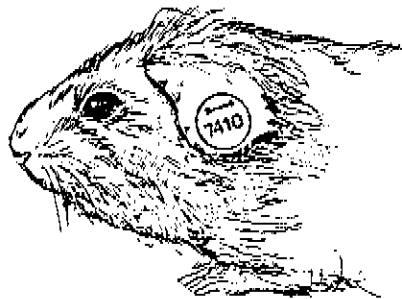


FIG. 7

1.2.3 Tattooing: this requires special instruments. The numbers or letters are inscribed in ink, usually on the inside of the ear. Such identification is permanent.

1.2.4 Rings: rings, which are generally of metal with inscription in clear, are used for rabbits. They are put on the hind leg above the tibio-tarsal joint. Small breeds of rabbit are ringed at 6-7 weeks, and large breeds at 9-12 weeks.

2. Methods most commonly used for the different species

The choice of method depends on:

- species;
- age;
- purpose of research:
 - permanent or temporary identification;
 - individual or group identification.

	Stains	Ear-punching	Description of markings	Tagging	Tattoo	Leg-ring
Mouse	x	x				
Rat	x	x				
Guinea-pig	x	x	x	x	x	
Rabbit	x	x	x	x	x	x (diameter depends on size of animal)

CHAPTER 4. RECORDING OF DATA

The rational management of an animal unit requires awareness in particular of:

- the numbers of the various categories of animal;
- reproduction performance;
- the filiation of individuals.

The data recording system should be devised to match the aim in view: e.g., management, selection, maintenance of strains. Depending on the system selected, this will require:

- a method of identifying each animal (Chapter 3);
- a cage record (label) on which the occupants are identified and on which the birth of new litters and any observations relating to experiments or other matters can regularly be recorded;
- permanent documentation from which genealogies are prepared and in which reproduction performance or characteristics are noted;
- day-to-day documentation (animal inventory).

1. The cage record; label (Annex 1)

The label identifies the occupants of a cage or a run.

1.1 Material: usually cardboard of various colours for easy identification of the categories of animals:

- breeding animals - strain - experimental group
- weaned young - generation.

1.2 Information

The label bears all requisite information on the occupants of the cage or run: e.g., number, sex, date of birth, as well as information on experiments where appropriate.

1.2.1 Breeding animals in pairs (1 male + 1 female) or isolated female:

- strain;
- identification mark or number;
- date of birth;
- date of mating;
- date set for sacrifice;
- date of birth of litters;
- total number of young;
- number of weaned young (males, females).

1.2.2 Breeding animals in a permanent polygamous unit: (several females + 1 male) (Annex 2)

Reproduction performance data cannot be given for individual females:

- strain;
- number of female and male breeding animals;
- identification number;
- date of formation of the unit (mating);

- date set for sacrifice;
- date of birth of litters;
- number of young;
- date of weaning;
- number of weaned young (males and females).

1.3 Attachment

The label is generally set in a label holder, a metal plate with lateral folds into which the label can be slotted. The label holder can be fixed to the cage permanently or temporarily.

1.4 Recommendations:

- When cages are changed make sure that the label is moved to the appropriate cage.
- Markers such as coloured tabs can be slipped into the label holder to indicate:
 - operations to be conducted (such as weaning, changing cage);
 - observations (animals with diarrhoea, animal found dead, anomaly observed, etc.).

2. Permanent documentation

The objective determines the type and method of presentation of the data.

2.1 Maintenance of consanguineous strains - permanent couples

In order to keep a consanguineous strain, the first prerequisite is to establish the genealogy of each individual in the strain. This applies particularly to mice and rats.

2.1.1 Individual identity

The "identity number" usually contains three components which link the individual:

- to the previous generation: parents' mating number;
- to its parents: serial number denoting the litter to which it belongs;
- to its brothers and sisters: a letter or figure to denote each individual in a given litter.

Example: mouse number "23-7-b"

"23": 23rd mating of the generation (matings by each generation are recorded in chronological order)

"7": 7th litter of the mouse's parents,

"b": individual "b" of that 7th litter.

2.1.2 Registration: there are two procedures:

(i) Single-register system (Annex 3)

This register contains data on:

(a) The parents:

- identity numbers of the male and of the female;
- identification mark. It must be noted that for practical reasons the identification mark on the individual might not be the same as the identity number entered on a document;
- mating number (following chronological order of marking by parents' generation);
- date of mating;
- date of sacrifice.

(b) The litters:

- identity number consisting of the parents' mating number and the serial number of the litter in the sequence of litters produced by the couple.

Example: Litter No. "179-2"

"179": 79th mating registered for the parents' generation;

"2": 2nd litter of the couple:

- date of birth of litter;
- number of weaned young;
- allocation of letter or figure to each member of the litter;
- observations or remarks on each member of the litter.

(ii) Dual-register system (see Annex 4)

(a) Mating register. This includes:

- the parents: same information as in single-register system;
- the litters: this states simply
 - identity number of litter;
 - date of birth of each litter;
 - number of weaned young from each litter.

(b) Litter register:

- identity number, comprising parents' mating number and "serial number" determined by the chronological order of births of litters in one generation and not to the same pair as in the single-register system.

Example: litter "132.150" (see Annex 4)

"132" is the mating number of the parents (see "Mating Register", Annex 4);

"150" is the serial number of the litter in its generation (see Litter Register, Annex 4);

- date of birth of the litter;
- number of weaned young;
- allocation of letter or figure to each individual in the litter;
- observations or remarks on each individual in the litter.

2.2 Non-permanent polygamous units (the females are separated from the male and each female is isolated for giving birth)

The "individual record" system is used, together with a simplified system for allocation of the identity number. This system is used mainly for guinea-pigs and rabbits, and occasionally for rats.

2.2.1 Register for allocation of identity numbers

An identity number is given to each breeding animal by simple entry in a register according to the chronological sequence of matings.

2.2.2 Individual record cards (Annex 5)

These record cards are generally used for breeding females. They can be used for males also if information on their reproductive performance is required (for male rabbits, for example). They contain data on:

- identity number of female (or male);
- identification mark;
- date of birth;
- date of death or sacrifice;
- identity number of parents;
- identity number of mate;
- date of birth of litters;
- number of young;
- number of males and females weaned;
- observations.

These "record cards" can be used to calculate a "productivity index" for each female, which facilitates:

- elimination of poor breeders;
- selection for breeding purposes of the descendants of the females which perform best.

2.3 Permanent polygamous units (where the females are not separated from the male) (Annex 6)

This involves the use of a "unit record card" which contains:

- the unit number;
- the number of females;
- the number of males;
- the mating date of the unit;
- the date of elimination of the unit;
- the date of birth of young;
- the number of young;
- the number of weaned males and females.

3. Day-to-day documentation

In an animal unit the staff must:

- know how many animals of each category are available at any given time;
- analyse the overall statistics on unit performance, in order to:
 - adapt production to demand,
 - detect any drop in productivity or any nutritional deficiency.

It is recommended that the following be compiled:

3.1 A weekly return for each room, species or strain. This document shows the number of animals available (Annex 7).

3.2 A monthly summary return, whose analysis allows production to be adapted to demand (Annex 8).

CAGE/PEN LABEL FOR BREEDING PAIR OR ISOLATED FEMALE

STRAIN:					
IDENTIFICATION MARK:			DATE OF BIRTH:		
IDENTIFICATION MARK:			DATE OF BIRTH:		
DATE OF MATING:			TO BE SACRIFICED:		
DATE OF DEATH:					
LITERS					
DATE	BORN	WEANED	DATE	BORN	WEANED

ANNEX 2

CAGE/PEN DOCUMENT FOR BREEDING ANIMALS IN PERMANENT POLYGAMOUS UNIT

STRAIN: DATE OF MATING:			
NUMBER: IDENTIFICATION NUMBERS *			
FEMALES:			
MALES:			
TO BE SACRIFICED:			
BIRTHS		WEANED YOUNG	
DATE	NUMBER	DATE	NUMBER
* where appropriate			

PERMANENT DOCUMENTS - SINGLE REGISTER SYSTEM*

STRAIN: <i>AKR</i> MATING NO.: <i>179</i> DATE: <i>15.01.88</i> FEMALE NO.: <i>56.3a</i> IDENTIFICATION: <i>56</i> MALE NO.: <i>56.3c</i> IDENTIFICATION: <i>56</i> DIED/SACRIFICED: DIED/SACRIFICED:			
LITTER: <i>179.1</i> DATE OF BIRTH: <i>25.02.88</i> WEANED YOUNG a) ♀ b) ♀ c) ♀ d) ♀ e) ♀ f) ♀ g) ♂	NUMBER: <i>..7..</i> OBSERVATIONS <i>Sent to laboratory</i> <i>sacrificed</i> <i>breeding</i> <i>skin test</i> <i>skin test</i> <i>breeding</i> <i>sacrificed</i>	LITTER: <i>179.2</i> DATE OF BIRTH: <i>27.02.88</i> WEANED YOUNG a) ♀ b) ♀ c) ♀ d) ♂	NUMBER: <i>..4..</i> OBSERVATIONS
LITTER: DATE OF BIRTH: WEANED YOUNG	NUMBER: OBSERVATIONS	LITTER: DATE OF BIRTH: WEANED YOUNG	NUMBER: OBSERVATIONS
LITTER: DATE OF BIRTH: WEANED YOUNG	NUMBER: OBSERVATIONS	LITTER: DATE OF BIRTH: WEANED YOUNG	NUMBER: OBSERVATIONS

* In each strain a group of mating numbers are set aside for each generation.

e.g.: generation 1: Nos 1-100
 generation 2: Nos 101-200.

PERMANENT DOCUMENTS - DUAL REGISTER SYSTEM*

1. MATING REGISTER

STRAIN: C3H		DATE: 15.01.88
MATING NO: 132		
FEMALE NO: 2i:2a	IDENTIFICATION: 21	DIED/SACRIFICED: ...
MALE NO: 2i:2d	IDENTIFICATION: 21	DIED/SACRIFICED: ...
LITTER:	DATE OF BIRTH:	NUMBER:
132:150	15.02.88	4...
132:182	21.02.88	9...
132:260	13.03.88	8...
STRAIN: ...		DATE:
MATING NO: 133		
FEMALE NO:	IDENTIFICATION:	DIED/SACRIFICED:
MALE NO:	IDENTIFICATION:	DIED/SACRIFICED:
LITTER:	DATE OF BIRTH:	NUMBER:
.....
.....
.....

* In each strain a group of mating numbers are set aside for each generation.

e.g.: generation 1: Nos. 1-100
 generation 2: Nos. 101-200.

2. LITTER REGISTER

STRAIN: ... <u>C3H</u>	MATING NUMBER OF PARENTS: <u>132</u>	SERIAL NUMBER OF THE LITTER: <u>150</u> (in generation)
DATE OF BIRTH: <u>15.02.88</u>	NUMBER: <u>4</u>	
WEANED YOUNG	OBSERVATIONS	
a)	Breeding	
b)	Breeding	
c)	Sacrificed	
d)	Sacrificed	

STRAIN: ... <u>C3H</u>	MATING NUMBER OF PARENTS: <u>140</u>	SERIAL NUMBER OF LITTER: <u>151</u> (in generation)
DATE OF BIRTH:	NUMBER:	
WEANED YOUNG	OBSERVATIONS	

INDIVIDUAL RECORD CARD*

SPECIES:	Identity number		Identification mark		Identity		Date of	
					father	mother	birth	death
STRAIN:								
SEX:								
Date of mating	Identity number of mate	Date of birth of litters	Cage/run number	Number of young born	number of young weaned	REMARKS - OBSERVATIONS		

* This card may be used in the context of non-permanent, polygamous matings as an "individual record" for females which are isolated for birth. This type of card may be used also for male rabbits, for example.

Annex 8

MONTHLY SUMMARY RETURNS

MONTH	J	F	M	A	M	J	J	A	S	O	N	D	TOTAL FOR YEAR
(10) Total usable production (7+9)													
(11) Number of young produced per female/week (10:1)													
(12) Average number of young weaned in each litter (6:5)													
(13)* % mortality before weaning													

* This percentage is calculated from the data on the labels, individual record cards or unit record cards.

The survey covers only a proportion of litters born in the month (15-25% of litters).

CHAPTER 5. BREEDING

General considerations

It is difficult to give "normal" parameters for breeding for the different species since they are influenced to a very large extent by:

- the strain or race;
- environmental factors, such as:
 - (i) temperature, lighting, season, etc. (physical factors);
 - (ii) nutrition;
 - (iii) social factors such as the presence or absence of other animals.

The figures given for the various species are therefore mean values (see Table 1).

(a) Puberty

The stage of development where the animal reaches sexual maturity. The age at which puberty occurs depends on species and strain, as well as on climatic and nutritional factors.

Puberty manifests itself in:

- the opening of the vagina in the female;
- the descent of the testicles into the scrotum in the male of certain species (such as rat).

Laboratory animals are mated for the first time shortly after the signs of puberty appear.

(b) Estrous cycle

From puberty onwards, the genital tract in the female undergoes a series of cyclical and repetitive transformations, called the "estrous cycle", which consists of four phases: proestrus, estrus, metestrus and diestrus. The length of the cycle varies from one species to another.

(c) Estrus

It is important for the animal breeder to know when the female is in the estrus phase, since it is then that she can be fertilized.

- she is receptive to the male. This is the period of "heat";
- ovulation takes place. In the female of some species ovulation is:
 - "spontaneous" (rat, mouse, guinea-pig), while in others it is "induced", i.e. caused by coitus or any other stimulation, as in the rabbit.

(d) Postpartum estrus

In rat, mouse and guinea-pig, an estrus occurs a few hours after parturition. If the male is present, the female can be fertilized. This is very important from a practical point of view. An advantage of all "permanent" mating systems over the "non-permanent" systems is this possibility of fertilizing the female during the postpartum estrus.

(e) Pseudo-gestation

After copulation that does not result in fertilization there is no estrus, and therefore no ovulation, for a certain time. This is called "pseudo-gestation". During that period, the female is not receptive and cannot be fertilized. The length of pseudo-gestation varies from one species to another.

(f) "Controlled" mating

Male and female are left together until copulation and separated immediately after (rabbit).

(g) Vaginal plug

In some species the sperm coagulates in the vagina after ejaculation. The vaginal plug is solid, whitish and somewhat conical. This "plug" is rapidly eliminated in some species (rat, rabbit) and in others (mouse) may remain up to 24 hours. The detection of a vaginal plug in a female tells the handler that copulation has taken place but not necessarily fertilization.

(h) Vaginal smear

The estrus can be detected by vaginal cytology. The technique consists of taking desquamated vaginal cells in order to determine the proportions of the various types of cells.

Mating systems

Laboratory animals are mated in accordance with systems that depend on:

- the biological features of the species;
- the types of animal required (consanguineous or not);
- production requirements.

These systems are classed in two groups, each of which has two varieties:

"Permanent" mating: the female is not separated from the male at the time of parturition. Mating is effected in one of two ways:

- "monogamous" mating: one male and one female are put together and constitute a couple;
- "polygamous" mating: one or several males are put together with several females to form a breeding unit.

"Temporary" mating: the female is separated from the male during pregnancy, and birth takes place in the male's absence. Copulation is therefore not possible during the postpartum estrus.

- "Polygamous" or "harem" type: a unit comprises one or several males and several females. Each female is removed from the unit before giving birth and put back in the unit once the young have been weaned.
- "Monogamous": the female is isolated from the male before giving birth.

TABLE 1
MEAN BREEDING DATA

	Mouse	Rat	Guinea-pig	Rabbit	Time
Age at puberty:					
male	6-7	6-8	8	6-7	weeks
female	5-7	5-8	10	5-6	weeks
Age at first mating:					
male:	8	11-12	10-12	6-7	weeks
female	6-8	11-12	12-14	5-6	weeks
Length of cycle	4-5	4-5	16-19	12-16	days
Length of estrus	7-20 hours	9-20 hours	6-15 hours	12-16 days	
Time of ovulation	2-3 after beginning of estrus	8-11	10	10-13 after mating	hours
Postpartum estrus	within 28 hours		3-8 h.	---	after parturition
Length of pregnancy	19-21	21-23	62-72	31-32	days
Number of young per litter	10-12	9-11	3	7-9	
Length of lactation period	16-21 days	21 days	14-25 days	6-8 weeks	
Length of fertile period:					
male	1.0-1.5	1	5	1-4	years
female	5-8 litters	1	4-5	1-4	years
Average weight at birth	1.0-1.5	5-6	90-100	64	grams

Reproduction: mouse - rat - guinea-pig - rabbit (Table 1)

1. Mouse

1.1 Puberty

- female: opening of vagina at 28-49 days.
- male: maturation of spermatozoa at 45 days on average.

1.2 Estrous cycle

The estrous cycle is four to five days in length. It is heavily influenced by many factors, including:

- strain;
- social environment (suppression of the estrus can occur among females grouped in the same cage).

The estrus lasts 7-20 hours. This is the period of the cycle in which the female is sexually receptive and can be fertilized. The estrus phase can be detected by vaginal cytology. For the vaginal smear technique see Vol. I, Chapter 12.

1.3 Postpartum estrus

An estrus occurs 14-28 hours after parturition. This is very important in the mouse, for which only the "permanent" mating system is used. Fertilization during the postpartum estrus ensures very high productivity.

1.4 Ovulation

The mouse is a species with "spontaneous" ovulation. Ovulation takes place 2-3 hours after the beginning of the estrus.

1.5 Mating

Receptivity to the male is not limited to the estrus. The female can also be receptive during the proestrus and the metestrus. The vaginal plug remains for 16-24 hours after mating. After mating during the postpartum estrus the vaginal plug is smaller and more difficult to detect.

Checks for the vaginal plug are made in the course of controlled matings:

- to obtain "dated" embryos, i.e. when it is necessary to know the age of the embryos;
- for females which are to undergo caesarean section.

Depending on the strain, the proportion of pregnant females to those presenting a vaginal plug varies from 30% to 100%.

1.6 Gestation

Non-suckling females: the length of gestation varies, depending on strain, from 19 to 21 days.

Example: a greater proportion of the DBA/2 strain of mouse gives birth at 19 days than is the case with the C57BL strain.

Hybrids generally have a shorter gestation period.

Suckling females: when a female is fertilized during postpartum estrus at a time when she is suckling, the gestation period is prolonged since the implantation of the egg after fertilization is delayed. If the number of sucklings is 1-2, gestation is prolonged by 7 days on average, and if the number of sucklings is greater than 3, gestation may be prolonged by 12-16 days.

Variations in the length of gestation period according to strain must be known by those who perform caesarean sections.

1.7 Pseudogestation

Pseudogestation lasts 11 days on average. During that period the female cannot be fertilized.

- There is a certain amount of pseudogestation among females who are grouped together.

1.8 Parturition

Parturition takes place at random around the clock and is not influenced by the alternation of light and darkness. The duration of parturition depends on the number of young. In practice it runs from one to three-and-a-half hours for a litter of 11.

1.9 Lactation and weaning

Lactation lasts four weeks on average, though this varies from one strain to another. Milk production increases until the tenth day after parturition, then decreases until weaning.

The young begin to take solid food from the age of 14 days, but they cannot nourish themselves fully and independently before the age of 16 days.

- Make sure weaning takes place at 21 days.
- Put the males in different cages from the females.
- During weaning, never put more than 30 animals in the same cage, since they tend to huddle together in the corners, and may suffocate.

1.10 Mating systems

The first mating takes place at the age of 6-8 weeks for females, 8 weeks for males. For convenience, animals of the same age are often used for mating. Mice should not be paired off after the age of 12 weeks.

Breeding animals are sacrificed between the ages of 12 and 18 months. This gives an active breeding life of 10-16 months. Set an age for the sacrifice of breeding animals in accordance with strain, mating system and the conditions in the breeding unit.

"Permanent" mating is the method most often used in practice for mice. This permits fertilization during the postpartum estrus.

1.10.1 Permanent monogamous mating

One male and one female are kept as a pair throughout their reproductive life.

- This system must be used without fail for the maintenance of a consanguineous strain.

1.10.2 Permanent polygamous mating

Units are made up of one male and 2-6 females. The most commonly used system is the "threesome": one male and two females.

- This system must be used for bulk production.

2. Rat

2.1 Puberty

Female: opening of vagina between 28 and 60 days. The first estrus occurs one or two days later.

Male: puberty is characterized by the descent of the testicles into the scrotum and the appearance of the spermatogenic cycle. The age at sexual maturation is approximately 40 days.

2.2 Estrous cycle

The female rat has a cycle of 4-5 days.

To detect the estrus:

- use the vaginal smear technique (see Vol. I, Chapter 12)
- observe the behaviour of the female (this requires practice)
 - she becomes hyperactive
 - she adopts a hollowed-back posture when the male approaches or when she is stimulated manually in the dorsal region at the base of the tail.

Postpartum estrus: same as mouse

2.3 Mating

A vaginal plug can be observed when the animals have mated, though it is eliminated fairly quickly, after a few hours.

- To ensure that mating has taken place, check for the presence of spermatozoa (see Vol. I, Chapter 12).

2.4 Gestation

Non-suckling female: gestation lasts 21-23 days.

Suckling female: as with the mouse, gestation can be prolonged if the female is suckling.

Gestation can be diagnosed by:

- weekly weighing;
- palpation; in practice, diagnosis by palpation can be made from the thirteenth day of gestation onwards.

2.5 Pseudogestation

Average duration is 12-14 days, and during that period the female rat cannot be fertilized.

2.6 Parturition

As with the mouse, birth takes place at random at any time of day or night, and it lasts between one and two hours on average depending on the age of the mother and the number of young in the litter.

- The female should not be disturbed during the 24-48 hours following parturition.

2.7 Lactation and weaning

Lactation lasts 3 weeks on average. The young can feed independently, depending on the type of feed, from the age of 17 days.

- The young should be weaned at 20-21 days. Earlier weaning can delay growth.
- The same precautions should be taken as for the mouse.

2.8 Mating systems

The first mating takes place at the age of 11-12 weeks. Depending on strain and conditions (nutrition, mating system) the breeding animals are sacrificed at the age of 12 months on average.

2.8.1 Permanent mating

(a) Monogamous mating: rats are rarely kept in pairs.

- This technique should be used only for the maintenance of consanguineous strains.

(b) Polygamous mating: this system, where a male is kept together with several females, is relatively rarely used in practice.

2.8.2 Non-permanent mating: this technique uses only polygamous mating in one of two forms:

(a) System involving isolation of females: form groups of one male and 4-6 females. At the twentieth day after mating, isolate the pregnant females in individual cages. Put the females back together with the same male when the litter is weaned.

(b) System involving "rotation" of males: form groups of eight females with one male, with four cages each containing two females (Table 2).

The male is put for 2 weeks at a time in each cage of the group; he thus returns to the same cage every 8 weeks by rotation. In the course of those 8 weeks, each female will have time for one gestation period (3 weeks) and one lactation period (3 weeks). The young are weaned at the latest the day before the male is put back in the female's cage.

Example: Week 3. The male from group 1 is put in cage 2. After 2 weeks (week 5) he will be transferred to cage 3, etc.

This system whereby all the males of the various groups are transferred from one cage to another on the same day, every fortnight, makes it easier to run and supervise production programmes.

3. Guinea-pig

3.1 Puberty

Female: the vagina is usually covered by an epithelial membrane which disappears periodically during estrus and parturition.

The first rupture of the vaginal membrane takes place at the age of 58 days on average (33-111 days). The first estrus takes place at 68 days (33-130 days).

Male: at the age of 60 days on average.

3.2 The estrus cycle

Average length 16 days, depending on the strain and the individual animal.

Estrus: average duration eight hours (6-15 hours). The onset of the estrus can be observed as follows:

- in the behaviour of the female:
 - pacing the cage;
 - mounting the other females (like a male);
 - hollowed-back posture, which can be triggered by
 - the male rubbing its nose on the back and hindquarters
 - touching the animal's posterior
- by the disappearance of the vaginal membrane approximately one day before estrus. The membrane stays open for three or four days. Estrus can however take place without rupture of the membrane.
- by the vaginal smear technique.

Post-partum estrus: this takes place between three and eight hours after dropping. If the male is present, fertilization can take place. The percentage of fertilization depends on:

- strain;
- age;
- number of previous pregnancies;
- number of females per male;

five females/one male: 87% fertilization)
20 females/one male: 50% fertilization) on average

- diet, etc.

3.3 Mating

In the guinea-pig the vaginal plug is not always present after ejaculation.

- To be sure that mating has taken place, check for the presence of spermatozoa (see Vol. I, Chapter 12).

3.4 Gestation

Average duration is 68 days, and actual duration varies according to:

- the strain;
- the number of young in the litter;

Example: litter of five: 67 days; litter of one: 70 days.

Diagnosis of gestation: by palpation around the fifth week.

3.5 Pseudogestation: there is no pseudogestation in the guinea-pig.

3.6 Parturition

The newborn animals are very advanced in their development. Their eyes are open, their coats and teeth are developed, they begin to walk as soon as they are born and they start to take solid food within the first few days of life.

The pubic symphysis of the female dilates to ease the dropping of young.

- 24-36 hours before parturition gap in the pubic symphysis is 17-22 mm. This gap is measured to predict the time of parturition when a caesarean operation is to be performed at term.

3.7 The litter

Taking all strains together, the average size of litter is 3.5. Average weight at birth depends on the number of young in the litter:

- 3-4 animals: 75-80 g
- 1 animal: 140 g.

- Sacrifice the young whose birth weight is less than 50 g.

3.8 Weaning

After 21-28 days. In polygamous units where the exact age is not known:

- wean animals which have reached the weight of 170-180 g
- separate males from females in different cages or runs.

3.9 Mating system (Table 3)

The first mating takes place at the age of 10-12 weeks for males, 12-14 weeks for females.

- In practice, females weighing around 400 g should be mated with males weighing 500 g. The polygamous mating system is the one generally used for guinea-pigs.

3.9.1 "Non-permanent" mating

This system, whereby pregnant females are isolated for parturition, is a non-intensive method of production.

(a) Unit comprising 1 male and 4-6 females

The females are isolated during pregnancy in individual cages. When they have weaned their young they are returned to the unit with the same male (type 1 mating system, see Table 3).

(b) Unit comprising:

10 females	+ 1 male	
	or	
10-20 females	+ 2 males)
)
	or)
20-30 females	+ 3 males)

Each male in turn is put with the females for one week

Pregnant females are withdrawn from the "mating unit" and put along with other females in "maternity units" where young are brought up communally (type 2 mating system, see Table 3).

3.9.2 "Permanent" mating

The females are not separated from the male. Fertilization can therefore take place during the postpartum estrus. This system ensures maximum productivity from each female.

(a) Monogamous unit

One male is paired with one female for the duration of their reproductive life. This system is hardly ever used (type 3 mating system, see Table 3).

(b) Polygamous unit: This comprises one or more males and several females

10 females	+ 1 male
	or
10-20 females	+ 2 males, which are used alternately with the females for one week at a time.

The females are not isolated during pregnancy and may therefore be fertilized during the postpartum estrus. It is recommended that the number of females per unit should not exceed 20 (type 4 mating system, see Table 3). This is the most intensive breeding system.

TABLE 3

AVERAGE BREEDING FIGURES FOR EACH MATING SYSTEM USED WITH THE GUINEA-PIG*

	Non-permanent mating		Permanent mating	
	Type 1 Isolation of females in separate cages	Type 2 Isolation of females in maternity pens	Type 3 Monogamous	Type 4 Polygamous
Intervals between litters	15 weeks	17 weeks	9-1/2 weeks	10-1/2 weeks
No. of litters per annum	3.50	3.10	5.40	4.90
No. of young per litter	3.90	3.82	3.90	3.90
% weaned/born	93.90	86	90	84.70
No. of weaned young/female/ year	12.69	10.18	18.90	15.96
Age of female at sacrifice	27 months	27 months	27-30 months	20 months
Advantages	- Individual performance easily established	- Good use of available space; - communal rearing of young	- Use of post- partum estrus; - individual performance easily established; - very high productivity per female	- Utilization of postpartum estrus; - good use of available space; - good productivity per female
Disadvantages	- Postpartum estrus not utilized; - inefficient use of available space	- Postpartum estrus not utilized; - low individual productivity per female	- Inefficient use of space; - many males required; - increased production costs	- Difficult to establish individual performance

* Using the example of a strain which produces an average of 3.88 animals per litter.

4. Rabbit

4.1 Puberty

Females can copulate one or two months before the first ovulation. Age at puberty depends mainly on breed:

- small breeds (1.5 kg) 4 months
- middle-size breeds (3.5 kg) 4-7 months
- large breeds (5-7 kg) 9-12 months

Males: In spite of the presence of spermatozoa from the age of four months, puberty occurs at between six and seven months in middle-sized breeds.

4.2 Estrous cycle

Unlike female rodents, female rabbits do not have a definite cycle, although there is a certain rhythm of receptivity to the male.

Estrus: This part of the cycle is long, at between 12 and 16 days, and the other phases are very short. It is during the estrus that the female is most receptive to the male:

- the vulva is generally congested, although this is not always a sign that the female is receptive;
- the female is agitated and rubs her chin against the cage.

Ovulation in the rabbit is not "spontaneous" but must be "stimulated" in one of a number of ways, such as:

- contact with other females
- coitus

Ovulation takes place 10-13 hours after stimulation.

4.3 Mating

Female: If receptive, the female adopts a hollowed-back posture. Female receptivity depends on:

- the individual
- the physiological condition: females which are suckling, moulting or suffering from malnutrition often are unreceptive sexually
- circumstances: a female may refuse one male and accept another.

Male: After ejaculation the male quickly falls backwards or to the side emitting a cry. The ejaculate may form a vaginal plug which is eliminated shortly after copulation.

For breeding:

- Put the female in the cage of the male. Leave them together for 15 minutes. If they fight or if copulation does not take place try with another male or try again the following day.

- When the female is unwilling or the male inexperienced, human intervention can effect a "supervised mating". One hand holds the female still by the ears and the scruff of the neck while the other hand passes under the abdomen and between the hind legs with the thumb to the right of the vulva and the index finger to the left (from the handler's point of view) pushing gently upwards to flatten the tail against the back. The body is then supported by the hand and the hindquarters are presented in normal mating position to receive the male.

4.4 Gestation

Gestation lasts 31-32 days on average. In large breeds it lasts longer. Diagnosis is by palpation.

For palpation handlers should hold the animal's ears and the scruff of the neck in one hand while placing the other hand between the hind legs slightly forward of the pelvis. The thumb is placed to the right and the other fingers to the left of the uterine horns so as to palpate the fetus. At around 12-14 days, the fetuses are the size of marbles (20 mm in diameter). After the fourteenth day it is more difficult to distinguish the fetuses from the organs in the abdominal cavity (Fig. 1).

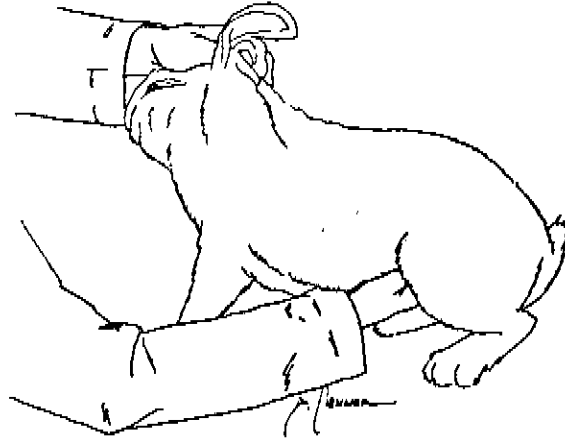


Fig. 1

4.5 Pseudogestation

This state results from ovulation triggered by stimulation such as:

sterile copulation or mounting of another female by the female concerned.

Pseudogestation lasts 16-17 days. During that period the female cannot be fertilized.

- Isolate the females that are to be mated at least 16 days before mating in order to avoid mating a female in a state of pseudogestation.

4.6 Parturition

The nest: During the last three days of gestation the female makes a nest with litter or food (hay or straw) and the hairs she tears from accessible parts (the abdomen and haunches).

Food consumption drops two or three days before parturition, which takes place very early in the morning and often goes unnoticed. Delivery of the litter takes approximately 30 minutes. Sometimes the last of the litter are born some hours or even days after the first.

4.9.1 Mating after weaning

The female is presented to the male when the litter has been weaned (at eight weeks), i.e. 56 days after parturition (or earlier if weaning is accomplished in less than eight weeks).

4.9.2 "Postpartum" mating (after parturition)

Suckling female: the female can be mated during the period of sexual receptivity following parturition. However, for this method the female must be in excellent physical condition and must receive a balanced diet. The conception rate is inversely proportional to the number of young being suckled by the female. This system is not recommended.

Non-suckling female: when the litter is gone or has been withdrawn, the period of sexual receptivity is extended to some 36 days after parturition. The female can therefore be mated once again during that period.

CHAPTER 6. FEEDING REQUIREMENTS OF VARIOUS SPECIES*

1. Mouse: the mouse is omnivorous.

1.1 Type of diet

In most colonies mice are given only "composite" food in the form of pellets approximately 18 mm in diameter and 25 mm long. Average daily consumption of food pellets is 4-5 g per adult.**

1.2 Water

Water is generally distributed in bottles provided with pipettes. Average daily intake is 20 ml/100 g of body weight.

2. Rat: the rat is omnivorous

2.1 Type of diet

The same as for the mouse. Average daily consumption of food pellets is 12-15 g per adult.

2.2 Water

Average water consumption is 10 ml/100 g of body weight.

3. Guinea-pig: the guinea-pig is herbivorous.

3.1 Type of diet

(a) Diet of "composite" food

It is difficult to feed the guinea-pig solely with "composite" food unless the diet is perfectly balanced. This food comes in granules approximately 5 mm in diameter and 10 mm long. Average daily consumption of food granules is 40-50 g per adult.

In practice fresh vegetables and hay are an important supplement to the guinea-pig's diet in spite of the risk of contamination they entail.

(b) "Farmer"-type diet

This diet comprises a mixture of ground cereals (2 parts) and bran (1 part). The food is slightly moistened and given to the animals in the form of paste in order to make it more palatable. Because of the danger of fermentation, food which is not consumed the day it is made up must be disposed of. The diet may be supplemented with fresh greens and hay ad libitum

* Specimen diet formulas for the various species are given in Volume I, Chapter 3.

** For all species the quantities of water and food consumed vary in accordance with the composition of the diet, temperature, humidity, physiological state and other factors. Young animals consume less food than adults, though as a proportion of body weight they consume more.

(c) "Mixed" diet

This is the most commonly used type of diet. It includes:

- (i) "composite" feed in granules 5 mm in diameter;
- (ii) green stuff: vegetables, grass, lucern, roots and sprouted grain. These are indispensable if the diet has no vitamin C supplement.
- (iii) hay: in the absence of hay, guinea-pigs eat the litter or the hairs of their neighbours (picking). Hay would therefore seem to be an indispensable ingredient in a well-balanced diet. Good quality hay (both graminaceous and leguminous plants) should be provided ad libitum. It is better to avoid the use of coarse hay that could damage the oral mucosa, leaving the way open for a number of infections.

The guinea-pig does not synthesize vitamin C, which can be provided in the following ways:

- added to the "whole" diet (200 mg of ascorbic acid per kg of diet)
- in the drinking-water (see Volume I, Chapter 3)
- in fresh vegetables

It should be borne in mind that fresh vegetables and hay are foods that involve a high risk of contamination for the animals.

3.2 Water

Average daily consumption varies from 10 to 40 ml/100 g of body weight depending on the amount of fresh food consumed.

4. Rabbit: the rabbit is herbivorous.

4.1 Type of diet

Unlike the guinea-pig, the rabbit seems to be able to cope with a diet based exclusively on "composite" feed, as long as it is perfectly balanced. Whatever diet is chosen, the rabbit requires a large quantity of cellulose for bulk.

(a) "Composite" diet

The ration should contain 12-15% cellulose. The granules are 5 mm in diameter. Average daily consumption is between 130 and 140 g per adult, and from 250 to 400 g for suckling or pregnant females.

There is a tendency especially among the male and non-suckling female animals to put on weight when food is available ad libitum; it is therefore necessary to ensure appropriate rationing:

- stop ad libitum feeding. Supply a measured quantity once a day.
- provide a "composite" diet with 25% cellulose.

If the "composite" food is not available or if its balance cannot be guaranteed, add fresh food and hay or use the "farmer" diet.

(b) "Farmer diet": same procedure as for the guinea-pig

(c) "Mixed diet": same procedure as for the guinea-pig

4.2 Water

Water consumption varies in accordance with the amount of fresh food in the diet. The average consumption of water (at 21°C) is 10 ml/100 g body weight for males and 90 ml/100 g body weight for suckling females.

4.3 General recommendations

- never put the food directly on the floor of the cage. Use troughs or containers;
- never distribute more food than is necessary to support the occupants of the cage or pen for a period of 3 or 4 days;
- unless instructions are given to the contrary (experiment, restriction in food intake) food and water should be supplied to the animals ad libitum;
- where possible avoid distributing fresh food which is often a major source of contamination;
- if fresh food is distributed, it should be changed every day;
- ensure that the watering system works properly;
- ensure that the animals always have fresh and clean water;
- in order to limit the proliferation of bacteria or algae, drinking-water may be acidified with hydrochloric acid at pH 2-2.5 (usually 2 ml of hydrochloric acid for every 2 litres of water; check the pH and adjust concentration);
- when possible use composite, sterilized food in pellet form (at least for rats and mice). In such cases no fresh food supplement should be given. Composite food can only be sterilized if it has been specially prepared to allow such treatment. In specific-pathogen-free (SPF) breeding, the use of sterilized food is imperative.

CHAPTER 7. LITTER

The term "litter" or "bedding" is applied to any absorbent substance used:

- In direct contact with the animal on the floor of cages or pens ("direct" litter),
- in litter trays for excrement under cages with holes in the floor ("indirect" litter).

The litter absorbs the humidity of excrement. The provision of nesting material is not indispensable. It does, however, enable the female to make a nest for the young, providing a microenvironment and adapting it to changes in temperature and humidity.

1. Litter

The choice of litter is often a delicate matter because of its influence on the animals' physiological reactions and because of pollutants or substances it may contain (insecticides or fungicides). Litter should therefore be chosen by the head of the unit.

1.1 Criteria

Good litter should be:

- absorbent: it should be capable of absorbing several times its own weight of liquid. Use that as a test to compare the absorbent qualities of litters;
- readily available: use a material that can be obtained all year round in the requisite quantities, and locally if possible;
- non-nutritional: whatever type of litter is used the animals tend to eat a certain amount of it. Unpalatable litter of low nutritional content is recommended;
- non-toxic (there should be no insecticides or fungicides especially);
- dust-free: the presence of very fine particles can irritate the respiratory tract and obstruct the nostrils of neonates (rats and mice);
- soft, with neither points nor edges that could cut;
- easily destroyed;
- low in price;
- free of insects and parasites.

The substances used as litter are generally industrial by-products and rarely possess all the qualities listed.

1.2 The products used

1.2.1 Sawdust

Soft sawdust is preferable. The sawdust from some exotic woods such as teak and mahogany contains aromatic substances which may be harmful.

Ensure if possible that the sawdust is packed at the workbench itself in order to obviate contamination by wild rodents, cats or dogs.

1.2.2 Wood shavings

As with sawdust, the wood shavings should be soft. This material is not very absorbent. It can nevertheless be used for mice.

1.2.3 Ground maize cobs

Very absorbent litter.

1.2.4 Ground sugarcane pulp and shells of groundnuts

The animals readily eat this.

1.2.5. Grain husks

The husks of cereals such as wheat and rice can be used, although the bristled awn of barley should not be used. Such material is only slightly absorbent, but it is cheap.

1.2.6 Cut straw

This poorly absorbent is used over a layer of sawdust in guinea-pig pens. The animals eat some of the straw.

1.2.7 Cotton

The by-products of the cotton industry are very absorbent and can be used as "direct" litter. Use only short fibre products.

1.2.8 Mineral litter

This absorbent manufactured product is not recommended for use as "direct" litter. It is used especially in trays under cages with wire-mesh floors. This material is expensive and cannot be incinerated.

2. Nesting material

Useless for guinea-pigs, not indispensable for rats and mice, but recommended for rabbits.

- strips of paper, wood fibre: for rats and mice
- hay: for rabbits.

3. Recommendations

- Choose a product which is locally available.
- Ensure that the source of the litter entails the least possible risk of contamination from dog, cat or wild rodent faeces.
- Sterilize the litter if facilities are available. If an ordinary autoclave is used, make sure that the steam penetrates to the centre of the loads (the autoclave should be able to make a high vacuum). Sterilized litter must be used in specific-pathogen-free (SPF) breeding.

- Store the litter in a place that is safe from wild rodents.
- Limit the thickness of the "direct" layer of litter to 2 or 3 cm. If the level of the litter reaches the opening of the drinking-water supply it may absorb the contents of the bottle.
- The level of "indirect" litter in the tray should not reach the wire-mesh floor of the cage.
- The litter should be changed regularly in accordance with the established routine (see Chapter 9).
- Soiled litter should be collected and removed (see Chapter 9).

CHAPTER 8. STERILIZATION AND DISINFECTION:
PREMISES, EQUIPMENT AND SUPPLIES

1. Use of disinfectants

1.1 Conditions of use

The way in which a disinfectant is used can alter its action, making it either more or less effective.

1.2 Duration of contact

The speed of action varies greatly from one disinfectant to another, although effectiveness generally increases with the duration of contact. The material should be immersed in the disinfectant solution for 1-2 hours.

1.3 Temperature

The use of hot solutions generally increases the efficacy of the disinfectant.

1.4 Age of solution

Many disinfectants are unstable in solution. Freshly prepared solutions must be used. The disinfectant solutions in germicide tubs and foot baths should be changed frequently to maintain their effectiveness.

1.5 Pollution level of the solution

Deactivation of the disinfectant by organic substances considerably reduces its efficacy. Disinfectant solutions in tubs where materials are soaked should be changed when a certain level of pollution is reached. The solutions in germicide tubs and foot baths in particular should be changed very regularly to keep them effective.

1.6 Contact with germs

The surfaces to be disinfected must always be washed first in order to remove all organic or other debris that could shield any germs and to prevent the organic substances from deactivating the disinfectant.

1.7 Compatibility

Certain substances can neutralize a disinfectant. Before using a disinfectant in conjunction with soap or detergent or any other product, the user must check that they are compatible.

1.8 Concentration

The disinfectant should be used in the concentration stipulated by the manufacturer. Regular use at lower concentrations may gradually give rise to resistant strains of microorganisms.

2. Procedure for disinfecting premises by fumigation

2.1 Formol

Formaldehyde is the most commonly used gas. This is a relative toxic, irritant gas. Every precaution should be taken to ensure the safety of staff. The animals should be removed from the premises before disinfection takes place.

Preparation of surfaces: dust and debris must be removed before disinfection. Walls, ceiling and floor should be washed with a bactericidal detergent solution such as "Tego".

This physical cleaning may be supplemented by treatment with an ammonia solution. This eliminates bacteria spores, coccidia oocysts and the eggs of parasites (Syphacia) which are hardly affected by formaldehyde fumigation.

2.2 All doors, windows, vents, etc. must be blocked. Use adhesive tape to keep them airtight.

2.3 Production of formaldehyde: the gas can be prepared in three ways:

- by heating formol in a receptacle placed in the room (commercial 40% solution of formaldehyde stabilized with methanol alcohol); 30-50 cm³ of formol per m³. The heating apparatus must be switched on and off from outside the room;
- by mixing formol with potassium permanganate in a receptacle; the reaction produces formaldehyde. 20 g of potassium permanganate and 30 cm³ of formol per m³ to be treated;
- by heating s-trioxane (powder) at a dosage of 5 g/m³. The heating apparatus must be switched on and off from outside the room.

The formaldehyde works best when room temperature is 24°C at a relative humidity of 80% (spread water on the floor).

2.4 Ventilate or air the room after 24 hours of fumigation; in order to avoid any risk to personnel the ventilation or airing must be activated from outside the premises.

2.5 Do not introduce the animals until 48 hours after ventilation.

3. Cleaning and sterilization or disinfection of equipment

The equipment (cages, troughs, bottles, etc.) can be treated in a variety of ways depending on the means available to the animal unit.

(a) Steam sterilization (see Volume I, Chapter 4) in a pressurized vessel (autoclave) is the most reliable method. It should be used wherever possible.

(b) Automatic washing: many suitable types of machine are available for the washing of all equipment.

- "tunnel" machine: a receptacle on wheels or rollers carries the material inside the machine where it is subjected to the action of sprays positioned appropriately for the material concerned. During the cycle the material is pre-rinsed, treated with detergent (at 80°C), rinsed, and rinsed again in hot water (90°C). The heat compounds the effect of the mechanical wash.

- Autoclaves and washing machines are fairly expensive pieces of equipment which many animal units cannot use.

(c) Steam treatment at atmospheric pressure: the maximum temperature that can be reached is 100°C. A number of machines (of the "Karcher" type) spray pressurized steam on to the material, adding mechanical action to the action of the heat. For use mainly on cages and racks.

(d) Spray treatment: the spraying of a disinfectant solution.

(e) Immersion treatment in a disinfectant solution. This method is often the most frequently used for the treatment of cages, bottles, tubes and small pieces of equipment, since it requires no special equipment apart from tubs. The efficacy of such disinfection depends on a number of factors:

- (i) The choice of disinfectant(s) and the instructions on their use are matters for the attention of an appropriately qualified person.
- (ii) The availability of tubs in the premises where material is washed. These tubs (of cement or metal) should be large enough to accommodate the most bulky pieces of equipment.
- (iii) Procedure for disinfection by immersion:
 - To ensure good contact with disinfectant, brush the equipment vigorously with a detergent solution, eliminating all traces of litter, food, faeces and urine. Bottles and pipettes can be cleaned with a bottle brush.
 - Rinse with running water.
 - Immerse the material in hot disinfectant solution at the recommended concentration for one to two hours (prolonged contact makes an important contribution to effective disinfection). Change the solution when the level of pollution by organic matter is considered to be high.
 - Rinse to remove all trace of the product used.

Note: Rabbit and guinea-pig urine in particular is alkaline (pH 8.2) and forms phosphate and carbonate crystals that leave a deposit of fur on the cage which is difficult to remove. The same is true to a lesser extent of rats and mice. Descaling agents with an acid pH ("Ceptolid", "Lime-A-Way") are recommended. Follow instructions for use.

4. Sterilization methods applicable to food

4.1 Humid heat (see Vol. I, Chapter 4)

4.2 Ethylene oxide: The food can be packed in airtight plastic wrapping and sterilized directly thanks to the penetrating power of ethylene oxide. A mixture of 10% ethylene oxide and carbon dioxide (CO₂) is used in the appropriate type of autoclave. The cycle involves:

- initial vacuum,
- intake of gaseous mixture while maintaining a partial vacuum to prevent diffusion to the outside. Maintain the interior temperature of the autoclave at approximately 40°C for six hours,
- evacuate the ethylene oxide by vacuum pump,
- flush with sterile air.

This type of sterilization has a long cycle and is used relatively rarely.

4.3 Dry heat: Use a tunnel oven heated by gas or electricity. The speed of the conveyor belt can be adjusted to provide the passage time desired. This method can be used for the manufacture of sterilized food in the form of biscuits which are directly sterilized.

- Prepare a flour from very finely ground ingredients and mix.
- Knead with water to form a dough.
- Mould to the requisite shape.
- Put the raw biscuits through the tunnel oven, regulating the temperature and length of passage to suit requirements. All combinations are possible. 150°C for 15 minutes is satisfactory.

4.4 Ionizing radiation

This can sterilize food which is packed in airtight plastic. Gamma radiation is used in general (cobalt 60 or caesium 137). Doses vary, depending on desired results and products to be treated, between 1.7 and 5 mrad. This sterilization technique is relatively rarely used as yet because of its high cost.

5. Sterilization of litter

Litter can be sterilized with:

- humid heat (see Vol. I, Chapter 4);
- ethylene oxide: in a steam autoclave equipped with vacuum pump,
 - initial vacuum,
 - intake of steam with 20% ethylene oxide,
 - maintenance at a relatively low temperature, 60°C for at least four hours,
 - vacuum, drying,
 - air before use.

6. Germicide tubs

Materials or supplies which cannot take heat treatment and are required for an SPF unit are sterilized in germicide tubs.

Examples of disinfectant solutions for "germicide" tubs

Tego 103G	1%
Formol	1%
Water	98%
Formol	20% (30% solution)
Benzalkonium (quaternary ammonium)	5% (50% solution)
Water	75%

It must be remembered that the efficacy of germicide tubs depends on frequent changing of the solutions since accumulation of organic debris inactivates them to a considerable extent.

7. General recommendations

In a "protected breeding environment" (SPF), supplies (such as food and litter) must be sterilized before they are introduced. An autoclave is therefore essential.

In an "open" (conventional) animal unit an autoclave is most useful, though not indispensable. A variety of disinfection techniques can be used for the treatment of cages, troughs, drinking-water bottles and other equipment.

CHAPTER 9. ROUTINE ACTIVITIES IN THE ANIMAL UNIT

Whatever the activity of the animal unit - breeding, stock or experiment - a number of tasks must be conducted at regular intervals. It is recommended that for each of them the following be set out in writing:

- how often it should be done,
- for some tasks (such as the cleaning of bottles), the technique or method to be used.

1. Monitoring of environmental conditions

- Monitoring apparatus is desirable (maximum and minimum thermometers, hygrothermography). Temperature and humidity should be recorded daily;
- If lighting is controlled, check the timer.

2. Litter

There must be a programme for the changing of litter; it depends on:

- type of litter
- type of cage (solid bottom or tray)
- breed
- number of animals per cage

Litter is generally changed between one and three times per week.

Rabbits: Changing the litter every day reduces the incidence of coccidiosis.

Soiled litter is collected in a container with a lid or, better, put directly in a plastic or heavy paper bag to avoid further handling before disposal and incineration.

3. Cages

Cages should be changed at least once per month. Available equipment dictates the rate of change:

- number of spare cages.

Example: If the animal house has 400 mouse cages, approximately 100 cages should be changed each week.

If washing and disinfection or sterilization take place:

- 1 day per week: 100 spare cages are needed
- 2 days per week: 50 spare cages are needed
- 3 days per week: 33 spare cages are needed, etc.

- capacity of washtubs or autoclave,
- facilities for disinfection or sterilization.

In order that changes be made systematically, the cages should be given a coloured tag or some other distinctive mark to indicate the day or week in which they must be changed.

- each time the animals are moved, the cages in which they are placed must be sterile or disinfected. Ensure that the label showing the contents of the cage is transferred to the new cage;

- the wire mesh floor of rabbit cages should be cleaned at least once per week, because excrement gathers on the matted hair of the animals;
- litter should be changed when the cages are changed.

4. Bottles

Water bottles, stoppers and pipettes should be changed and disinfected or sterilized at least once a week.

Weekly routine

Day 1. Bottles, stoppers and pipettes are changed and disinfected or sterilized.

Day 3, 5, 7. Changing of water. The bottles are emptied, rinsed and filled.

Day 2, 4, 6. Topping-up of water bottles.

After day 1 the bottles should be put back in the same cage in order to avoid spreading infection to animals in other cages. The water in the bottles should be changed daily if this is feasible.

When equipment cannot be sterilized, the bottles, pipettes and stoppers must be washed then immersed for at least an hour in a hot disinfectant solution (e.g. 1% "Tego" solution) (see Chapter 8).

Animals' drinking-water should be drawn straight from the mains. Never use water from a storage tank.

Water (especially acidified water) may be distributed for convenience in a plastic container of about 60 litres capacity set on wheels. This allows the bottles to be filled at each cage. The neck of the container is fitted with a tap. The water in such containers must be changed daily (see Vol. I, Chapter 2, Fig. 4).

5. Troughs

- filling (see Chapter 6)
- cleaning and disinfection (see Chapter 8).

6. Litter containers

When the containers are not in use their lids should be closed. Cleaning and disinfection take place once a week (1% "Tego" solution).

7. Food containers

- When rations have been distributed, the food must not remain in the same room but should be returned to the food store, which should be kept closed;
- Containers must be cleaned and disinfected before they are filled. Never refill a container in which there is old food or remains of food.

8. Cage racks

The cages should regularly be removed from their racks, which should be washed with a bactericide solution.

- If the racks can be dismantled they should be disinfected at least twice a year by immersion, pressurized steam or, if possible, autoclave.
- If the stands are mobile the castors should be regularly lubricated.
- The walls behind the racks should be washed frequently to remove dust.

9. Scales

- The dish of the scales and the weights should be carefully washed at least once a week.
- When scales are removed they should be reset.

10. Cleaning utensils

Brushes, shovels, buckets and cloths should be washed once a week with disinfectant solution.

11. Fixtures and movable equipment

Tables, washbasins, electrical fixtures, plumbing, door handles, etc. should also be given regular maintenance.

12. Footbaths

In order to reduce the risk of contamination from shoes or trolleys coming from outside, the entrance to the premises sometimes has footbaths or absorbent rugs soaked in a disinfectant solution. The efficacy of this measure depends on very frequent changing of the bactericide solution.

13. Floors, walls and ceilings

- Floors should be dusted daily with a damp cloth containing bactericide solution, or with a vacuum cleaner if possible. Sweeping the floor with a broom is not recommended.
- The floor should be washed at least once a week with detergent and disinfectant in hot water.
- The walls and ceilings should be washed once a year with a suitable detergent and disinfectant solution (such as "Tego" or quaternary ammonium).

14. Working clothes

It is imperative that the personnel of the animal unit have appropriate attire:

- before entering the premises, staff must put on the working attire specified by the head of the unit;

- overalls, trousers and headgear should be changed at least once a week and more often if necessary;
- shoes, sandals and boots should be cleaned with a disinfectant solution at least once a week;
- the staff in SPF breeding units generally take a shower before putting on their working clothes (see Vol. I, Chapter 14).

15. Inspection of cages, pens and boxes

Cages, pens and boxes should be inspected regularly and at least once in two days for the following purposes:

- noting births,
- weaning animals,
- removing dead animals: each dead animal should be sealed in a disposable plastic bag to which a form containing information on the animal is attached (Annex 1). Depending on instructions, the animal is either:
 - sent directly to the medical inspection unit; or
 - stored in a refrigerator or freezer until an examination can be conducted.
- checking animals for clinical signs of infection. All animals presenting symptoms or any abnormality should be reported to the medical inspection unit.

Look for:

- | | |
|------------------------|------------------|
| - prostration | - conjunctivitis |
| - ruffled fur | - dyspnoea |
| - loss of appetite | - skin lesions |
| - diarrhoea | - etc. |
| - running eyes or nose | |

The cages can be inspected when litter is changed, between 1 and 3 times per week.

FORM ACCOMPANYING EVERY ANIMAL SENT TO THE MEDICAL INSPECTION UNIT

REQUEST FOR EXAMINATION	
(To be completed by the animal unit)	Reference No:
DATE OF SENDING:	SPECIES OF ANIMAL:
ORIGIN:	STRAIN:
ROOM NO.:	AGE:
CLINICAL SIGNS OBSERVED:	OBSERVATIONS:
STUDIES REQUESTED	

EXAMINATION RESULTS	
(To be completed by the examining laboratory)	Reference No:
ACCOMPANYING MATERIAL:	
DATE RECEIVED:	
RESULTS:	

Example of an examination request form. It should be completed in duplicate and should accompany the animal. One copy is kept by the examining laboratory and the other is returned to the animal unit to communicate the results.

CHAPTER 10. MATING SYSTEMS FOR THE
MAINTENANCE OF CONSANGUINEOUS STRAINS
AND THE PRODUCTION OF CONSANGUINEOUS ANIMALS

Definition

"A strain is regarded as consanguineous when it has been maintained by mating brother x sister for at least 20 consecutive generations. Parent x offspring matings can substitute for brother x sister mating provided that the younger of the two parents is used for such mating."

1. Maintenance of a consanguineous strain

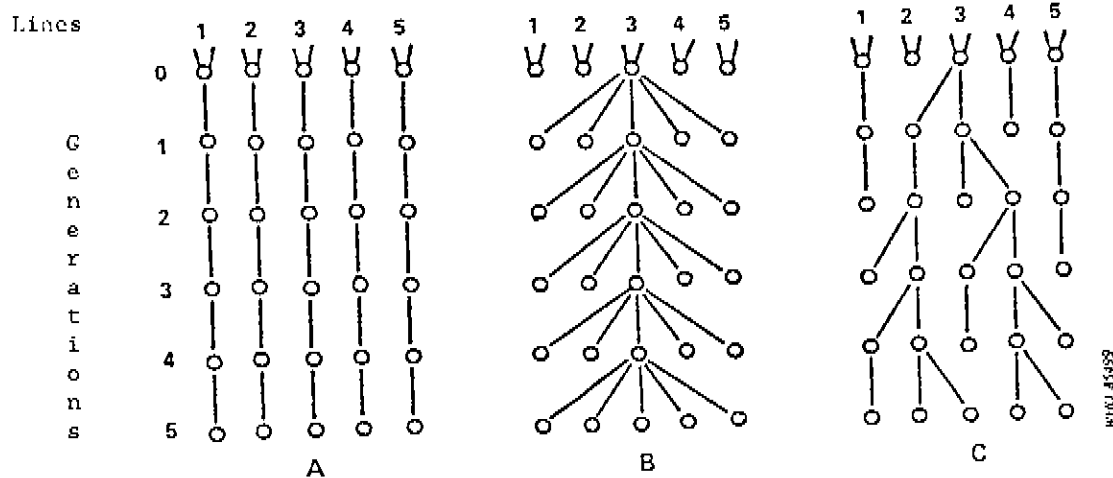
The colony, often referred to as a "consanguineous nucleus", which is used for maintenance of a consanguineous strain, always comprises a restricted number of animals.

1.1 Mating systems for maintenance of a "consanguineous nucleus"

The consanguineous strain must always be maintained by means of brother x sister mating. The head of the unit may choose either of the two systems in use:

1.1.1 Parallel lines system (Fig. 1, A)

FIG. 1*



* Each circle represents a brother x sister couple.

The stock at the outset might consist of five families (each family is descended from a single couple).

Generation 0: 1 brother and 1 sister are chosen from each family to make a couple. There are 5 couples in all.

Generation 1: Each couple in generation 0 contributes a family to generation 1. One brother and 1 sister are chosen from each family to make a total of 5 couples.

This is repeated for each generation. Each generation therefore has a total of 5 brother x sister couples from different families, and they are the parents of the following generation.

1.1.2 Single-line system (Fig. 1, B)

This is the system of choice used to maintain a consanguineous line.

Generation 0: 1 brother and 1 sister are chosen from a single family of the original stock to make a couple.

Generation 1: The generation 0 couple provides a family. From that family one brother x sister couple is chosen. The same procedure is followed from one generation to the next. With the single-line system, only one brother x sister couple in each generation gives birth to the following generation.

1.1.3 Mixed system (Fig. 1, C)

In practice it is often expedient to use a system which combines the previous two since, with the parallel-line system, certain lines become extinct after a number of generations, and they can be replaced from another line with the single-line system. The two systems can therefore alternate down the generations to overcome the difficulties encountered.

Example: In the five-lines system:

- line 2 provided no descendants. It was replaced in generation 1 using a couple from line 3.
- line 4 provided no further descendants for generation 2. A couple in line 3 provided replacements.
- lines 1 and 5 became extinct and were replaced from other lines.

Thus at the fourth and fifth generations, in spite of alternation between systems, the entire colony is descended from a single couple from generation 0, couple 3.

1.2 Some hard and fast rules

1.2.1 Allocate an identity number to each individual used for breeding (see Chapter 4).

1.2.2 Make a permanent mark on each animal with an identity number. The marking code generally used for mice consists of holes and notches punched in the ears (see Chapter 3).

The number denoted by the mark need not necessarily be the "identity number", as long as there is a chart that shows the relationships.

Example: A mouse which is registered with the number "79-1-b" might have the number "52" marked on its ear.

1.2.3 Enter each breeding animal and each litter in a genealogical register in accordance with the system adopted (see Chapter 4).

1.2.4 Avoid genetic contamination. The accidental introduction of even one breeding animal from another strain causes "genetic contamination". A strain which is genetically contaminated can no longer be considered consanguineous. It must be eliminated and replaced. To avoid genetic contamination it is recommended:

- not to keep different strains with the same colour of coat in the same room. If this is not possible, ensure at least that strains of the same colour are kept on different racks;
- to sacrifice every animal found in the room outside a cage;
- never to have two cages open at once on the same work-bench.

1.2.5 Do not differentiate strains genetically into "substrains": renew each consanguineous strain every five to 10 generations if possible, using animals from breeding units that can be considered "reference centres".

2. Production of animals of consanguineous strain

In order to ensure that consanguineous animals are produced in sufficient number, a "production colony" should be produced from couples in the "consanguineous nexus".

Consanguineous animals produced for experimentation should not be more than six generations removed from a common pair of ancestors. The production of consanguineous animals takes place in a number of units, and consists of three stages:

First stage

- Take one identified brother x sister couple from the "consanguineous nexus" (Fig. 1).

Second stage

- "Primary colony": form a "primary colony" from this couple by mating brother x sister (the process used for maintenance of a "consanguineous nexus") for two or three generations;

Third stage

- Production colony (Fig. 2). In the production colony no brother x sister matings are carried out, but animals of the same generation are mated. For practical reasons the various generations are differentiated by cage labels of different colours.

Generation 0: these are the parents of the "primary colony". Use white labels on the cages. The animals produced in this generation are used to produce generation 1 which will produce animals for experimentation.

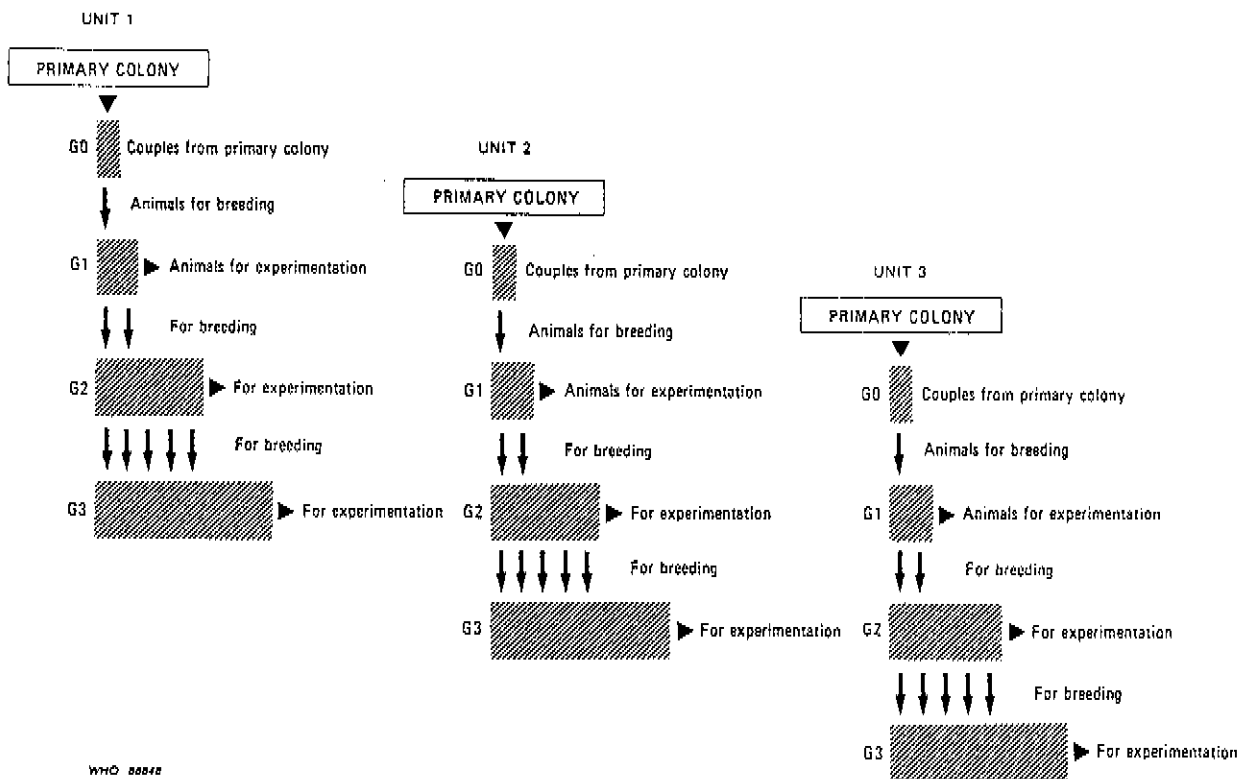
Generation 1: choose breeding animals from the young produced in cages with white labels. Use green labels for the cages of this generation. The animals produced in this generation can be used for both experimentation and reproduction.

Generation 2: as previous generation. Use yellow cage labels.

Generation 3: choose breeding animals from the young produced in cages with yellow labels. Use red labels on the cages. The animals produced in this generation may be used for experimentation only. At this generation the unit provides no more breeding animals. The generation is eliminated.

The production colony is regularly renewed with breeding animals from the primary colony which are used to produce units at intervals of a generation in order to ensure that there is never a shortage (Fig. 2).

FIG. 2. COLONY FOR PRODUCTION OF CONSANGUINEOUS ANIMALS



CHAPTER 11. MATING SYSTEMS FOR THE PRODUCTION OF NON-CONSANGUINEOUS STRAINS OF ANIMALS

In non-consanguineous colonies (such as Swiss mouse and Wistar rat) consanguinity must be avoided at all costs in order to maintain the characteristics of these types of colony, their prolificity in particular. It is generally agreed that the rate of consanguinity should not exceed 1% per generation.

The choice of mating systems to avoid consanguinity depends on the number of breeding animals (not on the total number of animals in the colony).

1. When the number of breeding males exceeds 100: breeding males and females are:
 - chosen at random
 - mated at random.
2. When the number of breeding males is less than 100: a system must be chosen for:
 - the choice of breeding animals,
 - mating

2.1 1 to 24 breeding males: use a system which reduces consanguinity to a minimum. This system may use pairs or "threesomes", but it is imperative that each male and female breeding animal have its pedigree, since each sire must provide a sire for the following generation and each breeding female must provide a breeding female for the following generation.

Example: Colony of 8 couples. Each couple is placed in a cage.

Generation 1: The couples are numbered from 1 to 8.

- The young keep the mating number of the parents until they themselves are paired off for the following generation.

Generation 2: The new couples are numbered 1 to 8.

- It is essential to take one male and one female from each couple in generation 1 to form these new couples.

No. of couple* of generation 1 producing the new male breeding animals of generation 2		No. of couple* of generation 1 producing the new female breeding animals of generation 2	<u>Generation 2</u> Serial No. of new couples* of generation 2
Male 1	x	Female 2	1
Male 3	x	Female 4	2
**Male 5	x	Female 6	3
Male 7	x	Female 8	4
Male 2	x	Female 1	5
Male 4	x	Female 3	6
Male 6	x	Female 5	7
Male 8	x	Female 7	8

* or threesome if mating is in threes.

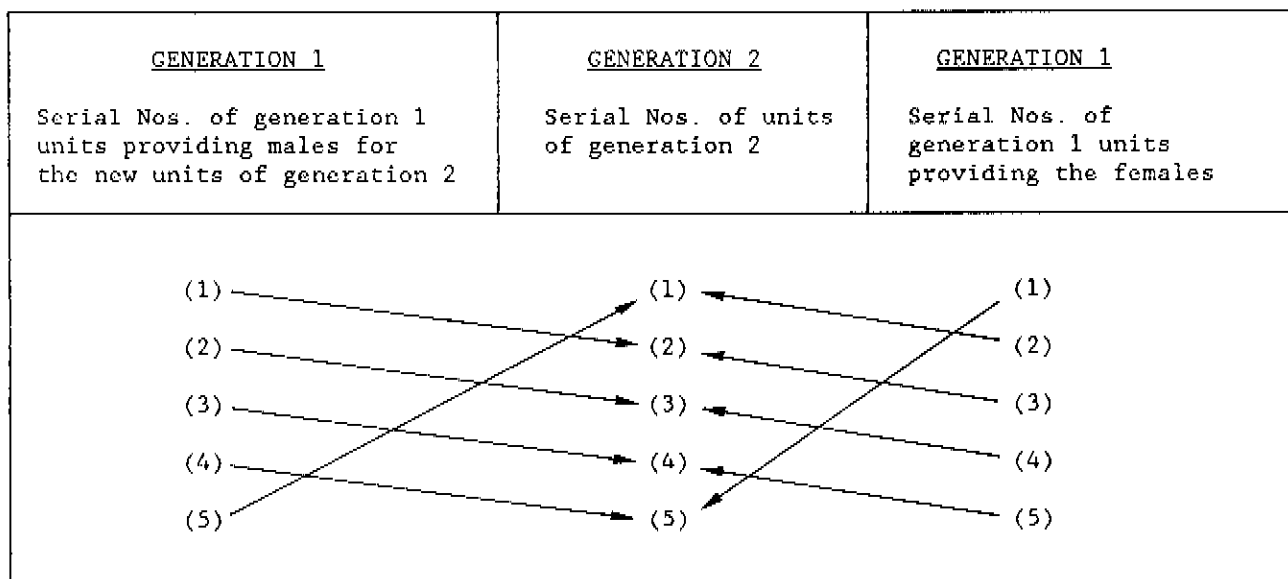
** Example: A young male from couple number 5 of generation 1 will be paired with a young female from couple 6 of generation 1. This new couple, which constitutes generation 2, will be numbered 3.

2.2 Number of male breeding animals between 25 and 100: "rotation system". The colony is divided into "units". Each unit can be one group of cages (2, 3, 4, ... cages).

Generation 1: the units are numbered 1, 2, 3, etc.

Generation 2: the new units are:

- numbered 1, 2, 3, etc.
- founded with young breeding animals from the units of generation 1 bearing different numbers, in accordance with the following plan:



Example:

- A colony of mice comprises 60 cages, each of which contains 1 male and 2 females: a total of 60 males and 120 females.
- This group of cages is divided into 5 "units" of 12 cages each. The "units" are numbered from 1 to 5.
- The new threesomes of generation 2 are made up as follows:
 - young males of unit 1 parents are mated with young females from unit 3 parents. These new threesomes will be allotted to unit 2 of generation 2.

CHAPTER 12. EUTHANASIA

The sacrifice of laboratory animals is necessary:

- at the end of an experiment: to conduct the examinations called for by the experimental protocol;
- in the course of breeding to eliminate:
 - excess animals
 - old breeding animals
 - animals which are sick or thought to be sick.

The term "euthanasia" designates any method which, quickly, painlessly and without stress, renders the animal unconscious and ends its life.

Whatever technique is used the following precepts must be observed:

- lift the animal gently taking care not to frighten it needlessly;
- remove it from the animal room;
- never perform euthanasia in the presence of other animals.

1. Criteria for the choice of technique

1.1 The choice will be made by the veterinary surgeon or the head of the unit, with due regard to:

- the animal: species, size, age.

1.2 The reasons for which the animal is to be sacrificed

- if the experimental protocol requires post-mortem examination, as is usually the case, ensure that the technique chosen entails no alterations which might distort the results of morbid anatomy examinations and histological, haematological, biochemical tests, etc.
- if the animal is to be sacrificed simply for the purposes of elimination the choice of technique is easier.

1.3 Safety of technique for the handler.

2. Various methods of euthanasia

2.1 Physical methods

2.1.1 Dislocation of cervical vertebrae (severe traction on medulla oblongata): method used for small animals:

- mouse
- rat: under 250 g
- guinea-pig: young
- rabbit: less than 1 kg

The technique requires a certain amount of practice.

(a) Mouse, rat, guinea-pig

Place thumb and index finger on either side of the neck at the base of the skull, immobilizing the animal against a hard surface. With the other hand grasp the tail or hindquarters, and pull so as to dislocate the cervical vertebrae from the skull and to separate the spinal cord from the brain.

(b) Rabbit

Grasp the head with one hand and the hindquarters with the other. A rapid pulling and twisting on the neck while the animal is stretched out dislocates the cervical vertebrae.

2.1.2 Chop (rabbit punch): technique used only for rabbits.

With one hand hold the animal by the hindquarters, head downwards. With the edge of the other hand deliver a sharp chop to the base of the animal's skull.

2.1.3 Advantages and disadvantages of these methods

Advantages:

- quick
- cost nothing
- no substance that might interfere with post-mortem examinations is introduced into the body.

Disadvantages:

- some find these methods repugnant and difficult to use;
- the tissues of the cervical region and possibly even of the brain cannot be subjected to histological examination.

2.1.4 Other physical methods

- Decapitation: by guillotine, for the rat
- Electrocution: 115 volt current
- Air injection: 5-10 cm³ intravenous injection for the rabbit.

These methods are relatively rarely used.

2.2 Inhalation methods

These use:

- overdose of volatile anaesthetic
- gases.

Inhalation techniques are useful for species to which intravenous injections cannot easily be administered. In the interest of safety and efficiency, they are usually used in an airtight system (caisson, jar, etc.).

2.2.1 Volatile anaesthetics: in order of rapidity of action these are:

- halothane
- chloroform
- ether

Cotton wool soaked in the substance is placed in the airtight container (caisson or jar) in order to create a saturated atmosphere. Do not allow the animal to come into direct contact with the product.

(a) Halothane:

Advantage:

- very fast-acting.

Disadvantages:

- expensive
- risk of hepatitis in man after repeated exposure.

(b) Chloroform: still very often used for euthanasia.

Advantage

- inexpensive

Disadvantages

- initially stimulant
- hepatotoxic
- must never be used in the proximity of mice because toxic for that species.

(c) Ether: the most commonly used.

Advantage:

- inexpensive

Disadvantages

- initially stimulant
- inflammable, explosive.

If animals are sacrificed with ether, care should be taken when storing and incinerating the carcasses because of the risk of explosion. For example, animals sacrificed by ether should never be put in a refrigerator which is not equipped with a fire-proofing system.

2.2.2 Gases

(a) CO₂ (carbon dioxide): very often used, inexpensive, rapidly induces narcosis, non-inflammable, and heavier than air, which reduces the danger of using it. Source:

- compressed gas in metal cylinders
- dry ice

The animals are placed:

- either in an airtight container as for ether and chloroform,
- or in a cage inside a sealed plastic bag.

(b) Other gases

- CO (carbon monoxide) less commonly used, efficient at a concentration of 2%, difficult to use. If an internal combustion engine is used to provide the gas, the carbon monoxide must be filtered and cooled to avoid irritating the animals.
- nitrogen: rarely used, expensive, does not induce narcosis and has no advantage over CO₂.

When using this gas care must be taken to avoid all danger to humans and the other animals. The operation must be conducted in separate premises with appropriate equipment (perfectly airtight).

2.3 Injection methods

These methods use:

- non-volatile anaesthetics administered in overdose
- other non-volatile substances.

Such products are generally injected intravenously. If another route of administration (intraperitoneal, intracardiac) is used, the anaesthetic effect of the first phase is less rapid. These substances should be administered under the supervision of a veterinary surgeon.

2.3.1 Non-volatile anaesthetics

The most commonly used are barbiturates:

- thiopental sodium ("Pentothal")
- pentobarbital sodium ("Nembutal")

2.3.2 Other non-volatile substances

The following substances are less frequently used:

- (a) Chloral hydrate, chloralose: little anaesthetic activity, death supervenes as a result of respiratory failure. These substances can interfere with study of the hepatic enzymes. They should be administered intraperitoneally.
- (b) Magnesium salts: magnesium sulfate is used in a concentration of between 2.5 and 4 ml/kg in an 80% saturated solution. This product is administered only intravenously. A barbiturate should be administered first to induce narcosis.

Advantages:

- inexpensive
- no interference with study of enzyme systems.

Disadvantage:

- spectacular reactions: convulsions, defecation, vocalization.

2.3.3 Dosage

Lethal effect is generally obtained:

- for anaesthetics with three times the dose used for anaesthesia;
- for other substances with a dose which is twice the LD₅₀ (median lethal dose).

LETHAL DOSES OF SOME SUBSTANCES ADMINISTERED BY INJECTION
(mg/kg)

	Chloralose		Chloral hydrate		Pentobarbital		Thiopental	
	IV*	IP**	IV	IP	IV	IP	IV	IP
Route of administration								
Mouse	342		1	200	210	210	225	400
Rat	165		1	100	120	120	140	240
Guinea-pig			1	200	90	90	110	115
Rabbit	360			400	120	120	140	140

*IV: intravenous

**IP: intraperitoneal

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