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PATHOPHYSIOLOGY

Laboratory Exercises

Pavel Sobotka et al.

Pathophysiology
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Content

1/ Basic Methods

1.1 Laboratory animals

An experimental animal is each animal, which is subjected to research, e.g. even an animal observed in the wild for population study. Laboratory animal is a narrower concept.

It is just an animal with known genetic characteristics, physiological and others that is specially bred for experimental purposes. Such an animal is standardized in terms of nutrition and environment and remains for all generations in the areas of laboratory breeding.

1.1.1 Division of laboratory animals

A. By genetic characteristics

Basically we distinguish 2 elementary lines:

a) Isogenic animals, i.e. genetically defined, identical, e.g. inbred strain
   The animals are obtained by close breeding for more than 20 generations among siblings or parents and their offspring. They are phenotypically uniform.
b) Non-isogenic animals, i.e. genetically undefined strains, e.g. outbred strain
   It is a genetically heterogeneous population without crossing with individuals coming from different inbreeding.

B. By bacterial colonization

This corresponds to the conditions of breeding.

a) Conventional animals with undefined microflora which are kept in open breeding facility complying basic hygienic conditions.
b) Specified pathogen free (SPF) animals which do not contain specified pathogens. They are in barrier breeding facility.
c) Gnotobiotic, axenic animals – germ free (GF), which are obtained by sterile hysterectomy. They are bred in isolators.

1.1.2 The quality of animals

is substantially influenced by their living conditions, temperature, humidity, noise, alternation of light and darkness, and the quality and quantity of food. Repeated contact with the breeding house staff and the experimenter, so called handling, is also important. Man must avoid disturbing the animals by any undue traumatic manipulation such as handling the animals with forceps, etc. which may lead to defensive reactions or agressiveness in the animals.

Laboratory animals are often used for the elaboration of models. The biological model is a living system which enables us to reproduce normal or pathological conditions of another living system including that of man. The animal model of disease is either spontaneous (with naturally acquired disturbance or with genetic disposition) or artificial (with arteficially introduced disturbance or disease).

Animal models of diseases:

- Mutant animals – appear spontaneusly or induced artificially
- Transgenic animals – modified with genetic material from another species using the techniques of genetic engineering; they belong to genetically modified organisms (GMO)
- Knock-out animals – removed some gen – for study of its function which is then missing

1.1.3 Ethics of work with experimental animals

There is no doubt that experiments using animals are the main source of research in medical science. Nevertheless, there are some limits in place that protect animals from misuse. The first legislative measure appeared in 1876 in Great Britain. Today the European convention about the protection of vertebrates which are used for experimental and other scientific purposes exists. Also the Czech Republic issued a law for the protection of animals in 1992 (amended in 2013).Some world-wide organisations for the protection of animals, e.g. People for Ethical Treatment of Animals (PETA) or Animal Liberation Front (ALF) are occasionally misused for such criminal acts as destruction of laboratories, release of animals into the wild etc. In this respect it is necessary to point out that mankind also uses animals as a source of food, for hard labour in agriculture, for competitive sports, for furs etc.

Today much effort is given to the development of alternative methods to partially or completely replace laboratory animals. This idea is supported by Russel and Burche who propagated in their publication (The Principles of Humane Experimental Technique, 1959) the principles of 3 R, namely Reduce, Refine, Replace.

Reduce means to use minimal number of animals that are necessary for successful and perfectly planned and prepared research.

Refine means to provide gentle treatment of laboratory animals with maximal welfare and reduction of stress and discomfort. Physiologic and ethologic needs of animals must be taken into account (size of breeding cages, number of animals kept together, light/dark cycle length, room temperature etc.). Breeding facility and laboratory staff must follow the rules for appropriate handling of the animals. All surgical procedures must be performed in a fashion that minimizes invasiveness and pain during operation and adequate post-operative care must be provided.

Replace means to use some alternative methods in research instead of laboratory animals, when is possible.

Various sorts of alternative methods were developed for the purpose:

1. Exploatation of information database
2. The use of mathematical models and videoprogrammes
3. The use of lower organisms
4. The use of isolated organs
5. The use of tissue and cell cultures
6. The use of physical and chemical methods
7. Experiments on human beings

Although very useful, alternative methods do not reflect the complexity and regulatory mechanisms of the whole organism. With respect to this issue, experiments conducted on animals are, up to this time, irreplaceable.

In addition, the results of experimentation on animals are limited due to the differences in various species. Therefore there is much to be said for the long-accepted method of testing medications, chemical or diagnostic and operative methods on animals before they are used on man himself.

1.1.4 Some vertebrates used in experiments

Mice (*Mus musculus*). Used mostly in pharmacology, toxicology, genetics of mammals virology, oncology. Now, many mutant strains are obtained either by natural way or by gene manipulation. These mutant strains have a high importance for possible modelling of different pathological states. Breeding and feeding as in rats.

Rat (*Rattus norvegicus*). Usually Wistar albino, Sprague-Dawley or Long-Evans. The widely used laboratory animal for acute and chronic experimentation and practical training. Breeding in cages of glass or synthetic material. Commercially available food is enriched with fat, vitamin D and minerals.

Rabbit (*Oryctolagus cuniculus*). Suitable for acute and chronic experiments and for laboratory methods (estimation of pyrogens, serology). High vegetative reactivity is characteristic. Vaccination against myxomatosis is necessary. Feeding of oats, hay with the addition of carrots or turnips. Breeding in wooden or metal cages is possible, or outdoors.

Guinea pig (*Cavia porcellus*). Suitable for experiments in microbiology and serology. Does not tolerate high exposure to temperature. Food similar to rabbits but with a higher requirement of vitamin C.

Dog (*Canis familiaris*). Besides bastards preferably are dogs with standard phenotype and suitable character, e.g. beagle. Breeding in cages with running area, food should be enriched with milk and vegetables. Suitable for acute and chronic experiments.

Cat (*Felis catus*). Suitable for acute experiments in the sphere of nervous system and respiration. The friendly access of the experimenter is important. Basis of food is meat and milk with addition of pasta.

Monkey (*Simian*). Due to the evolutionary similarity with man they are especially suitable for neurophysiological research. Often used in virology.

Basic biological data of laboratory animals are presented in Table 1.2.1.

Table 1.2.1 Main biological data of laboratory animals

	Dog	**Cat**	**Rabbit**	**Rat**	**Mice**	**Guinea pig**
Pregnancy (days)	58–66	56–64	30–33	21–23	19–21	65–72
Chromosomes (number)	78	38	44	42	40	64
Rectal temp (°C)	38.3	38.6	39.2	38	37.4	38.6
Heart rate	70–100	110–200	200–230	260–400	500–600	130–190
Respiration rate	12–20	18–25	35–60	70–150	100–210	90–150
Blood pressure (mm Hg)	115/60	120/75	110/80	120/80	115/80	90/56
Erythrocytes (10^{12}/l)	4–8	6–10	4–6	5–11	6–12	4–6
Hemoglobin (g/l)	149 (120–180)	110 (80–140)	120 (80–150)	150 (120–180)	150 (100–200)	140 (110–170)
Leukocytes (10^{9}/l)	7–18	6–15	6–12	8–14	7–15	4–15
Thrombocytes	200–600	170–700	110–400	400–800	100–400	85–160
Glucose (mmol/l)	4.9	3–5	3.5–7	5–8	5	–

1.1.5 Manipulation with laboratory animals

Rat: Remove from the cage by the tail at the base quickly but do not terrify it. With the other hand press the animal against pad and firmly grab the skin on the neck and back so that it can not move and bite (Fig. 1.2.1, 1.2.2). For application of an injection we need another person. Holding the rat by the tail for a longer period of time enables it to rotate and this could lead to scalping of the tail, therefore we provide it support (forearm, pad).

Mouse: Catch by the tail. With the other hand press the animal to the pad and grab the skin on the neck. We then grab the tail with the third and fourth finger of the same arm and with the free other hand we can inject.

Rabbit: grasp the skin on the neck and back with both hands.

Guinea pig: is fearful, scrapes. Hold the animal around the neck on the dorsal side.

1.2 Laboratory protocol (report)

A laboratory report (protocol) should be elaborated for each experiment. These reports (protocols) should contain the main purpose (aim) of the experiment, brief description of the methods used and clear statement of the data obtained. This may be in the form of graphic recordings or numerical tabulations, or both. All recordings must be correctly and adequately labeled, so that they can be easily interpreted.

Each laboratory report (protocol) should be dated, the species of laboratory object specified (its weight, sex and age), the amount of anesthetic and the way it is administered.

A practical knowledge of writing these reports (protocols) will be an important support in both health service and scientific research, even though the protocol of our laboratory experiments is more substantial.

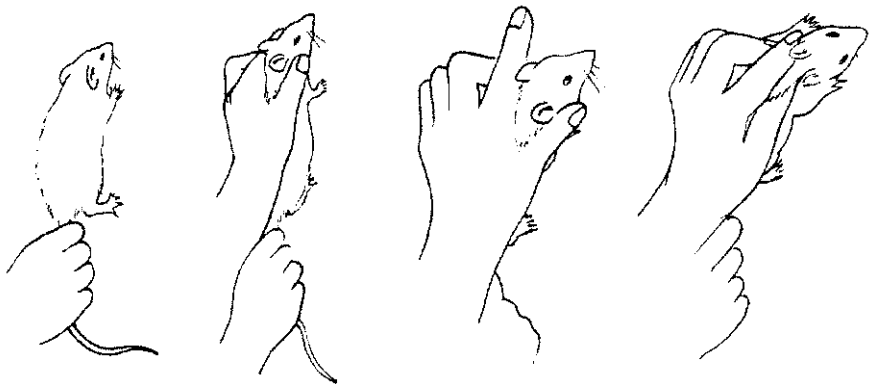

Fig. 1.2.1 Holding the rat with two hands

Fig. 1.2.2 Holding the rat with one hand