

Principles and ethics of experimental use of laboratory animals

Use of live systems

The idea of using animals in experiments

Claude Bernard (1813-1878) – physician



- 1867 publication *l'Introduction à l'étude de la médecine expérimentale* (Introduction to the study of experimental medicine)
 - „ *The principles of experimental medicine are the principles of all experimental sciences.*“

The most commonly used exp. animals

- laboratory mouse (*Mus musculus*)
- laboratory rat (*Rattus norvegicus*)
- laboratory rabbit (*Oryctolagus cuniculus*)
- golden hamster (*Mesocricetus auratus*)
- guinea pig (*Cavia apperea*)
- pig (*Sus scrofa*)
- African clawed frog (*Xenopus laevis*)
- fish - e.g. striped danio (*Danio rerio*)



Animal production systems for experiments

1. Open

- Free entrance of animals, persons and material, barrier-free

2. Closed

a) Barrier housing

- animals separated from the outside environment by a barrier and hygiene loop, not separated from humans
- SPF animals

b) Isolator

- animals confined in isolators, which separates them from the outside environment and humans
- axenic, gnotobiotic animals

According to housing method

– Conventional

- common facilities, open breeding and housing system, control of pathogens transmissible to humans

– SPF

- closed breeding and housing system, Specific Pathogen Free animals

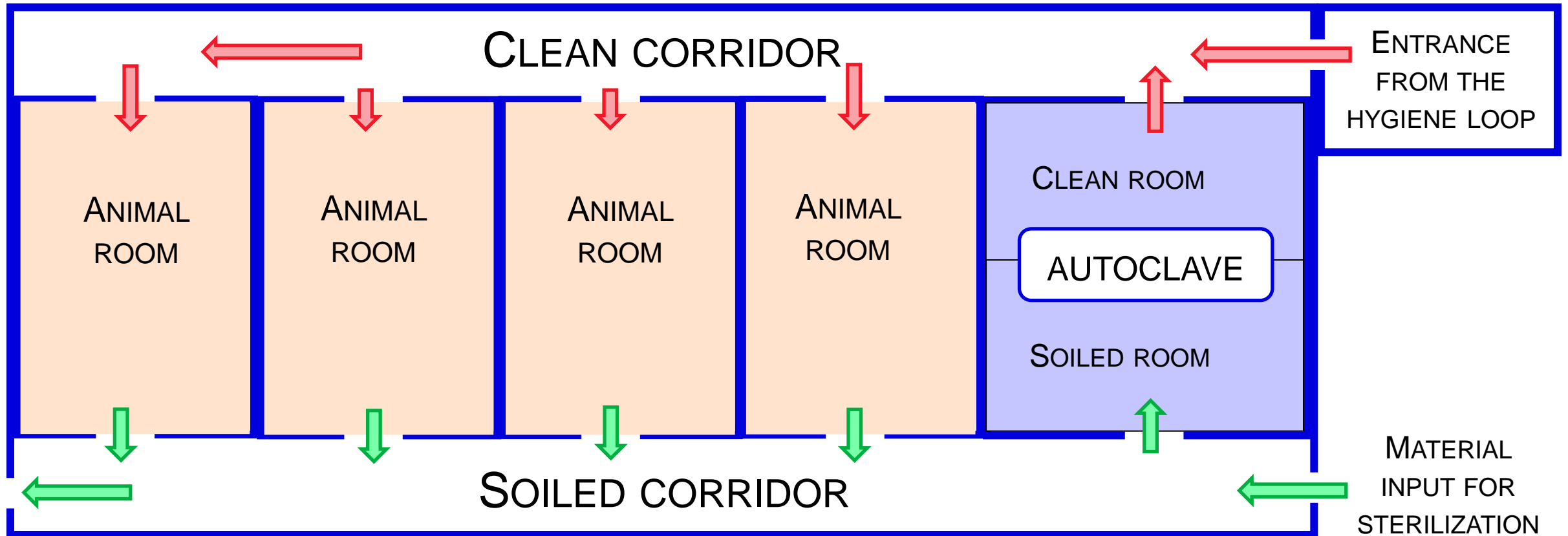
– Axenic

- closed breeding and housing system
- NO micro-organisms

– Gnotobiotick

- “*gnotos*” (known) and “*bios*” (life)
- closed breeding and housing system
- axenic animal + defined microorganism (mono-, di-, poly-xenic)

Barrier housing - scheme



Isolators



Animal models – a genetics perspective

Inbred

- colonies formed by at least 20 generations of brother-sister breeding that can be traced back to a single founding pair

Outbred

- crossbreeding colonies that have been geographically separated (different breeding facility) until they reach the same range of genetic variation

Hybrid

- by crossbreeding two inbred strains. This cross produces hybrid animals that are more resistant to disease, have higher survival rates under stress, live longer and have larger litters than the parent strains

Trangen

- used in almost every therapeutic and disease research area
- DNA modification (knock-out functional gene, knock-in a non-functional/muted gene, inserting a piece of foreign DNA, ...)

Animal models – immune system perspective

Immunocompetent

- no changes in the immune system

Immunodeficient

- animals with different levels of immunodeficiency and phenotypic characteristics

Humanised animal models

- induced human immune system of various levels

Using animals in the experiments

The 3Rs principle

- 1959 Russel a Burche
 - › Reduction
 - › Replacement
 - › Refinement

More information about the history of the 3Rs principles at:
https://norecopa.no/media/2ltdq3ht/the-three-rs_120324.pdf

Reduction

- any procedure that results in the same amount of information being obtained using fewer animals or maximising the information obtained from a single animal
- appropriate experimental design and adequate analysis of the resulting data, with due consideration of statistical principles, can increase the precision of the results and equally allow the use of fewer animals to develop the data
- a small pilot study will indicate whether or not it is appropriate to proceed to the main experiment

Replacement

- any experimental method that does not require the use of a whole, live animal
- some of these methods only partially replace
- humane killing of animals to obtain cells, tissues or organs for subsequent studies *in vitro*

Other methods substitute fully, as they do not require any biological material obtained from a fully developed vertebrate.

Animal experimentation alternatives

- Consistent mining of existing experimental data
- Models, computer programs
- Physico-chemical techniques
- Invertebrates, plants, microorganisms (lower organisms)
- Early developmental stages - bird egg germarium, embryos
- Human volunteers, epidemiological data
- Cell, tissue and organ cultures (relative surrogates - require freshly obtained cells or tissue)

Refinement

- modifying any procedure that interferes with the life of a laboratory animal from birth to death, so that the pain and suffering experienced by the animal is minimised and its standard of living is raised
- staff should be well trained and skilled in the handling of the species of animals used
- anaesthesia and analgesia should be used wherever appropriate and possible

Regulation of use of experimental animals

- Animal Protection Act No. **246/1992** Coll. (which is the implementation of Directive **2010/63/EU** of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes)
- Central Commission for the Protection of Animals (ÚKOZ)
- Expert Committee for Ensuring the Welfare of Experimental Animals

Expert Committee for Ensuring the Welfare of Experimental Animals

- at every supplier, breeder and **user facility**
- provides **consultancy** on the welfare of experimental animals, the 3Rs, ...
- establish, monitor and review internal operating procedures
- monitor and control the progress and results of experimental projects

Central Commission for the Protection of Animals (ÚKOZ)

- protection of livestock animals
- protection of pet animals
- protection of wild animals
- **protection of experimental animals**

Animal Protection Act No. 246/1992 Coll.

§ 3 Terms

a) an animal is any living vertebrate except a human being, but not a fetus or embryo,

...

j) experimental animal

1. a living vertebrate animal which is or is to be used for experiments, other than a human being, including self-feeding larval forms and mammalian fetuses from the last third of their normal development, or
2. a live cephalopod which is or is to be used for experiments;

an experimental animal shall also be considered to be an animal which is at an earlier stage of development than that referred to in point 1, if the animal is to be allowed to live beyond that stage of development and, as a result of the experiments carried out, is likely to suffer pain, suffering, distress or permanent harm when it reaches that stage of development,

Animal Protection Act No. 246/1992 Coll.

§ 18 Purposes of the experiments

(1) Experiments may be carried out for these purposes only:

- f) **higher education or training to acquire, maintain or improve professional skills**

Probator laparotomy

- animal model

Probator laparotomy



Hands-on

- 1) Animal in full surgical tolerance
- 2) Place the animal in the supine position it on the operating table
- 3) Laparotomy
- 4) Observation of the organs of the abdominal cavity
- 5) Suture of the muscle layer
- 6) Suture of the skin layer

Hands-on

- 1) Animal in full surgical tolerance
= under deep anaesthesia
- No further addition of anaesthesia
will be necessary



Hands-on

- 2) Place the animal in the supine position on the operating table
 - Use a rubber band to fix the thoracic and pelvic limbs
 - To fix the head, fasten the rubber band behind the upper teeth



Hands-on

3) Laparotomy

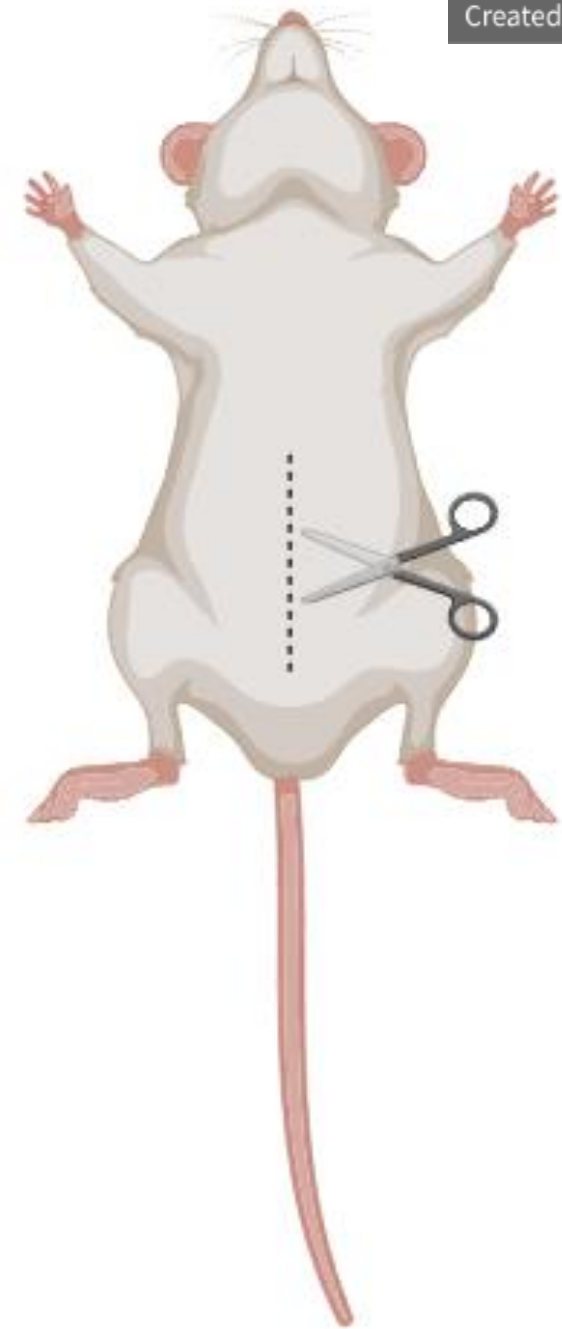
- Do a laparotomy in abdominal region.

1. Open the skin layer

2. Open the muscle layer.

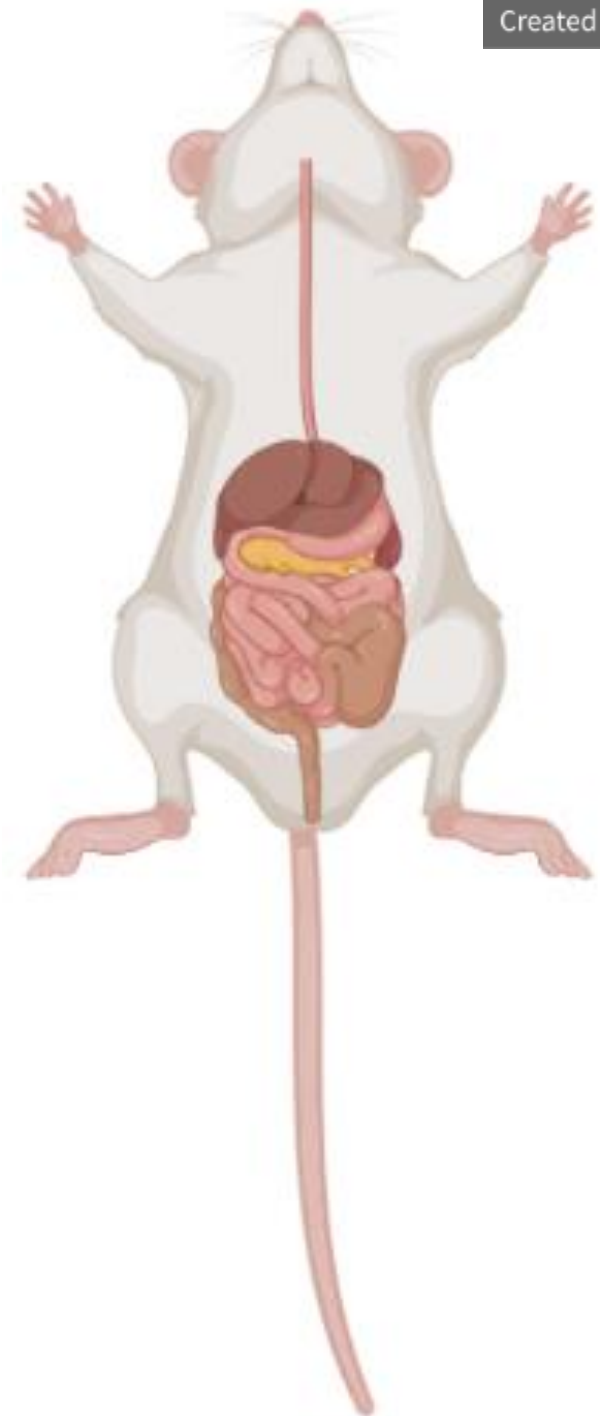
- The incision will run from the *processus xiphoideu* almost to the symphysis.

Be careful not to cut into the thorax!



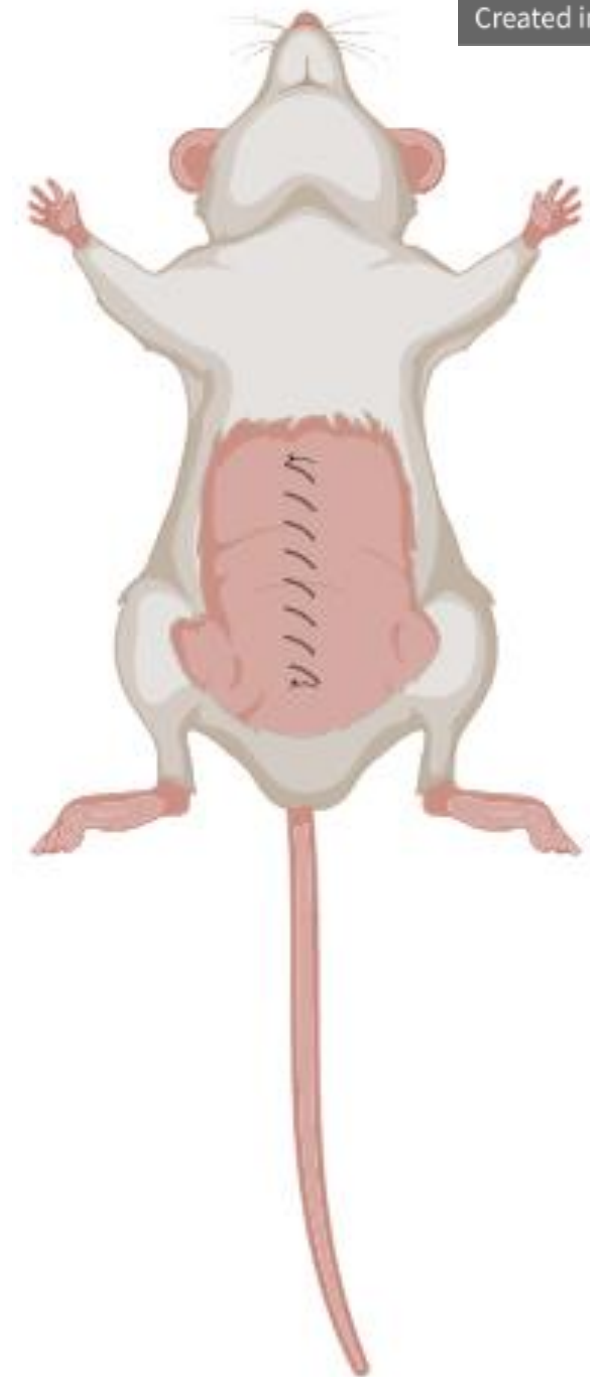
Hands-on

- 4) Observation of the organs of the abdominal cavity
 - caecum vs. Stomach
 - kidneys (both) + *vena renalis*
 - *aorta abdominalis* + *vena cava caudalis*
 - urinary bladder, ...



Hands-on

- 5) Stitching of the muscle layer
- Use of the continuation stitch
 - Round needle on the cross-section
 - Distance between stitches about 5-7 mm
 - Tighten the stitches properly so that the edges of the tissues fit tightly together
 - Do not over-tighten the sutures to avoid tissue ischemia (reducing healing)

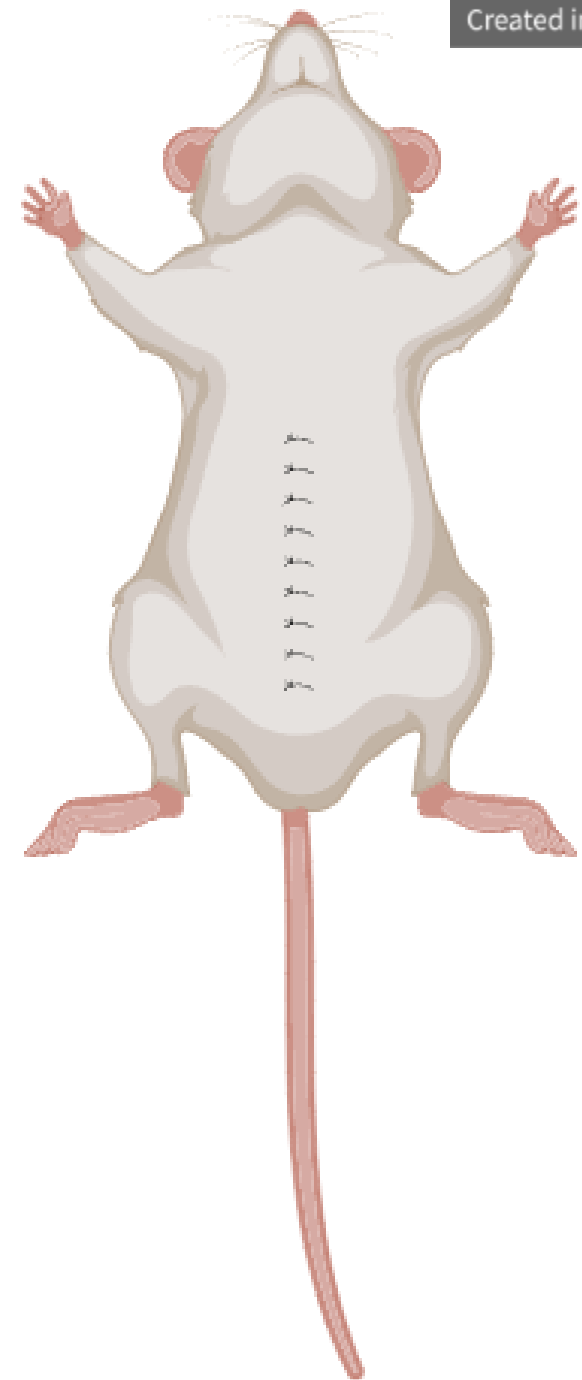


Hands-on

6) Stitching of the skin layer

- Use of individual stitches
- Triangular needle on the cross-section
- Distance between stitches about 5-7 mm
- Shorten the ends of the stitches to approx. 5 mm

- Tighten the stitches properly so that the edges of the tissues fit tightly together
- Do not over-tighten the sutures to avoid tissue ischemia (reducing healing)



Notes

If you become unwell (for any reason), do not worry and report this to the teacher immediately (to prevent an accident).

If the animal begins to move on its own at any time (showing signs of anesthesia wearing off; eg. move its tail, or raise its paw/s), report this to the teacher immediately.

**Eating, drinking and
smoking are forbidden in
the laboratory areas**



**It is forbidden to take
ANY records from
the laboratory areas**



M U N I

M E D