LABORATORY ANIMALS

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Importance of laboratory animals

- Physiology of animals
- Pathophysiology of diseases
- Development of medicaments and therapeutic approaches
- Verification of chemical substance safety, biocompatibility of materials
- Training of surgical techniques

Translation research

Importance for veterinary medicine (model X target organism)

Experiment X observation

Insulin discovery (1920, 1921)

- Frederick Banting, Charles Best, John James Richard Macleod

The 1st treated diabetic patient: Leonard Thompson 1922

The most important species of laboratory animals

- **Mouse** – most frequently used. Pharmacology, genetics of mammals, virology, models of human diseases (mutant strains, transgenic and knock-out mice)
- **Rat** – physiology of cognitive processes, behaviour, models of diabetes
- **Rabbit** – serology, insulin quantification, pyrogens quantification, tests of irritable effect of chemical substances on the cornea
- **Cat** – study of CNS and respiratory system
- **Dog** – e.g. beagle, use in electrophysiology, neurophysiology, pharmacology
- **Guinea-pig** – in microbiology and serology, physiology of the auditory system
- **Hamster** - genetics
- **Pig** – training of surgical techniques, temporary covering of burns with porcine skin
- **Primates** – rhesus monkey, baboon, chimpanzee – use in neurology, virology, behaviour

The most important species of laboratory animals

- **Frog** – Xenopus laevis, physiology of blood circulation, electrophysiology
- **Fish** - zebrafish (Danio rerio)
- **Molluscs**
- **Insects** - Drosophila melanogaster, genetics
- **Worms** - Caenorhabditis elegans

Genetics of laboratory animals

1. Isogenic = genetically defined strains

(isogenicity= genetic uniformity of all individuals)

2. Non-isogenic = genetically undefined strains

3. Genetically semi-defined strains

Isogenic strains

Inbred strains

- obtained by close breeding for more than 20 generations (brother + sister or offspring + one of the parents)
- homozygosity higher than 98 % (Degree of homozygosity is expressed as a coefficient of inbreeding.)
- features: isogenicity, phenotype uniformity (low variability of reactivity), usually low fertility, disposition to diseases
- advantages of use in experiment: homogenous statistical set, lower number of individuals is sufficient
- disadvantages of use in experiment: a risk, that the findings are strain-specific and are not valid for other strains, problematic generalization of the results

Coisogenic strains (mutant strains)

- differ from the original strain only in one gene, in which a mutation occurred

Isogenic strains

Congenic strains

- strains originated by cross-breeding of two strains and following back-cross-breeding with one of the original strains (at least 10 times, selection of some specific feature)
- specific genes of one strain on genetic background of the another strain

Recombinant-inbred strains

- crossing of 2 strains, the hybrids give origin of new lines which are then crossed brother x sister, what leads to establishing of a new strain

Rekombinant-congenic strains

- crossing of 2 strains followed with 3 back-crosses to one of the original strains and inbreeding with crossing brother x sister (at least 14 times)

Consomic strains

- a complete chromosome of one strain is transferred on the background of the second strain with back-crosses (similarly as for individual genes in congenic strains, but the process is more complicated)

Nonisogenic strains

Outbred lines

- genetically heterogeneous population without crossing with individuals coming from different, in the frame of the population close crossbreeding is avoided so that the coefficient of inbreeding remains as low as possible
- features: some level of phenotype variability (higher variability of reactivity), higher fertility and resistance to diseases
- advantages of use in experiment: cheaper and easier production, the findings have more general validity
- disadvantages of use in experiment: less homogeneous set, higher number of animals is necessary

Genetically heterogeneous lines

- originate by crossing of several inbred strains followed with breeding according to principles of outbred population

Outbred selected lines

- in an outbred population given phenotype feature is selected

Animal models of diseases

Spontaneous mutants X genetically modified organisms

Mutant animals

Transgenic animals

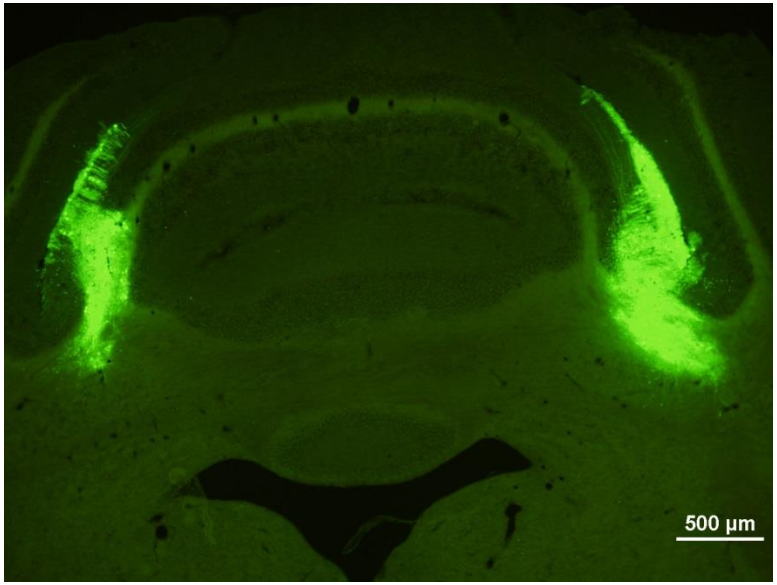
Knock-in

Knock-out (knock-down)

Conditioned mutants

Local transfection

Models on non-genetic principle



Gnotobiology of laboratory animals

Conventional animals

- undefined microflora
- open breeding facility complying basic hygienic conditions

SPF animals = specified pathogen free

- microflora of the animals certainly does not contain specified pathogens.
- barrier breeding facility

Gnotobiotic animals

- breeding isolators

1. **Axenic animals = germ free**
   - without any microbes
   - pups obtained with sterile hysterectomy or hysterotomy into sterile atmosphere of the isolator
2. **Asociated animals**
   - derived from axenic animals colonising them artificially with one or more species of microorganisms
   - monoxenic, dixenic, polyxenic

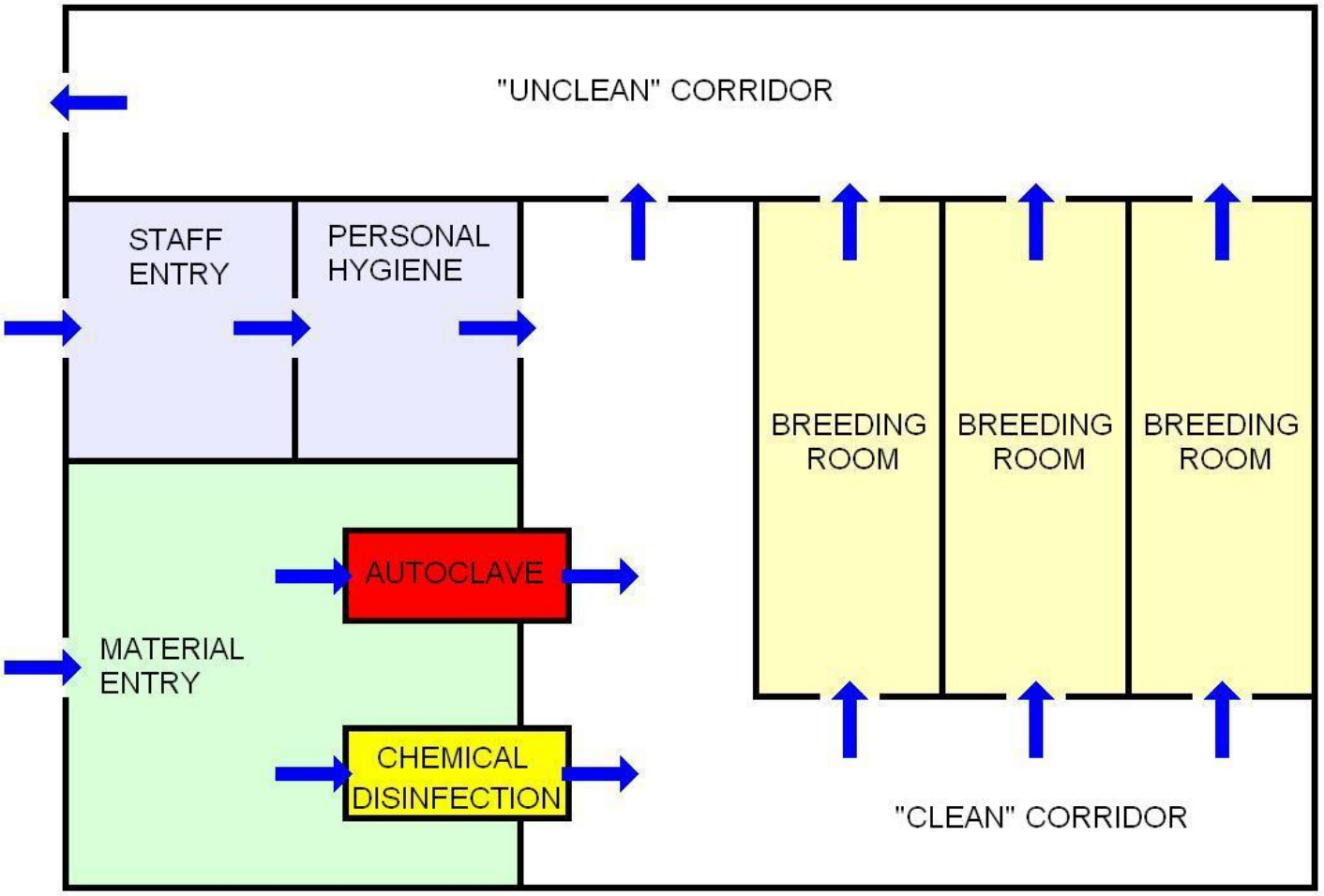
SYSTEMS OF BREEDING OF LABORATORY ANINMALS

Open – communication without the barrier

Barrier – the space with the animals id separated from external environment and movements of animals, people and material are controlled to eliminate possible introduction of alien factors from the external environment (infection) – sterilization of coming water, food, sawdust used for bedding, perfect personal hygiene of the personal.

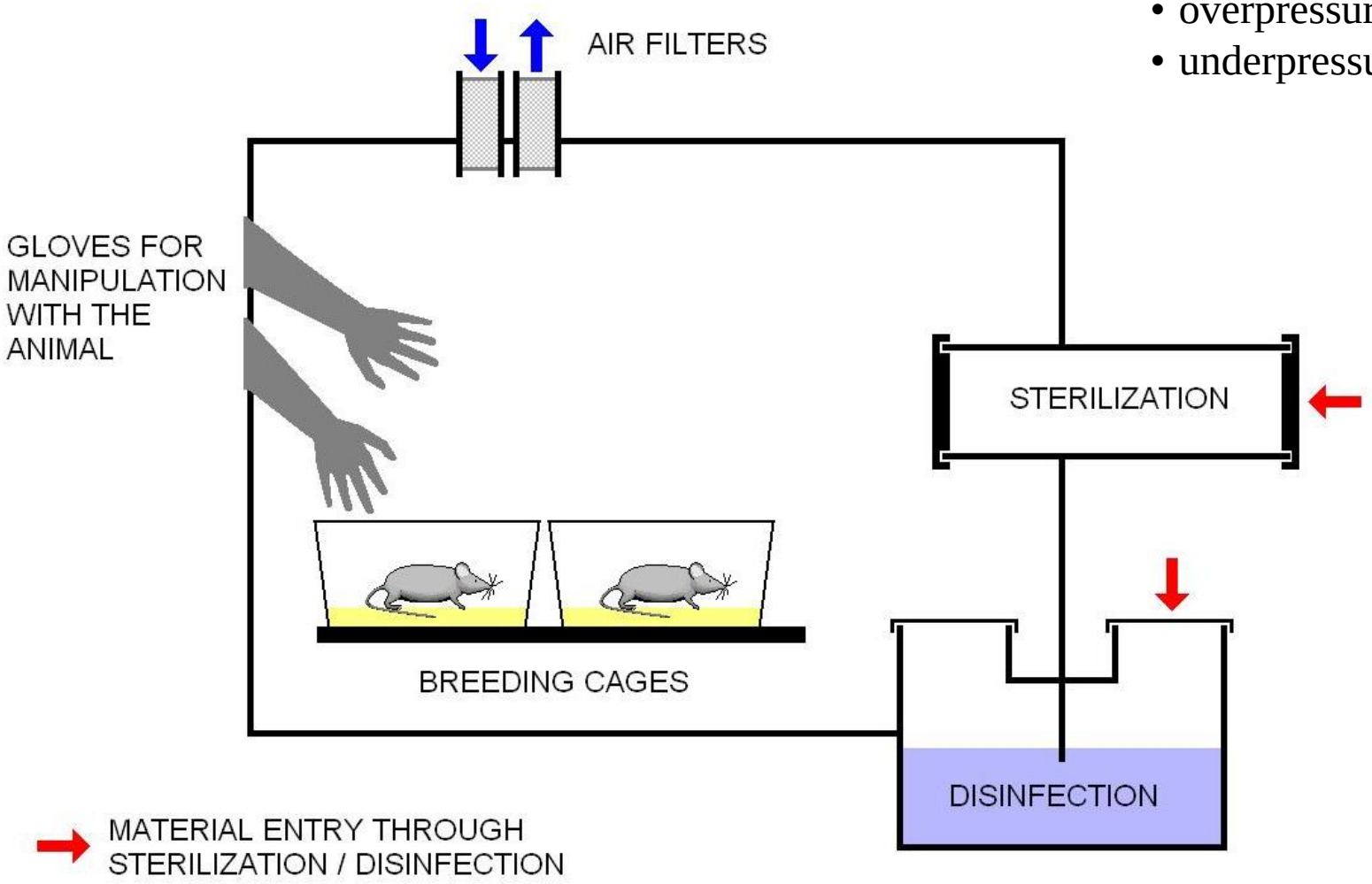
Isolator – the space for the animals is permanently separated by a barrier from external environment as well as from people who manipulate with the animals.

Scheme of barrier facility



Scheme of isolator

Isolators:
- overpressure
- underpressure



PRINCIPLES OF LABORATORY ANIMALS USE

3 R: REPLACEMENT

REDUCTION

REFINEMENT

Welfare, quality standardization

Breeding of animals for scientific purposes

Alternative tests

- Must undergo **validation procedure**, provided by ICCVAM (Interagency Coordinating Committee on the validation of alternative methods). Every new alternative method is peer reviewed by an international group of experts, who are not financially interested in the results. For some time both methods can be used simultaneously.
- According to ECVAM (European Centre for the Validation of alternative methods) **21 scientifically verified alternative methods** (namely tests of phototoxicity, skin irritability, embryotoxicity, pyrogenity) are approved at present time.

In vitro methods

Cell and tissue cultures

- plant, animal, human cells cultured in a laboratory
- examples:
  - ***Neutral Red assay*** – examined substance is added to the culture together with contrast dye which is absorbed by living cells only.
  - ***HeLa cells*** – an immortal cell line used in oncological research derived from cervical cancer cells taken from Henrietta Lacks 1951.
  - ***3T3 NRU phototoxicity test -*** tested substance is added to cell cultures in various concentrations, then UV radiation is applied
  - ***EYETEX screen test -*** protein solution prepared from beans – replacement of DRAIZE test of eye irritability. Destruction caused by the chemical leads to opacification of the solution.

Microorganisms

- example: ***Ames's test*** - test of mutagenicity, tested substance is added to agar with Salmonella typhimurium with defective gene for histidine synthesis. If the substance is mutagenous, caused reverse mutation and the bacteria became able to produce → bacteria survives. Number of bacterial colonies is proportional to mutagenous potential of the substance.

Computer and mathematic models (in silico methods)

- generalizing models, quality of the prediction depends on the quality of input data
- During making the model replacing use of laboratory animals, it is necessary to use numerous animals to acquire enough data for. Estimation which is derived from the model has to be verified by an experiment.

Tests in lower animals of lower ontogenetic stadium

- Chicken embryo – replacement of DRAIZE test; embryonic membranes are vascularized but without inervation
- Heddles – tests of LD50
- eggs of African clawed frog (Xenopus laevis) – tests of mortality, malformations, growth inhibition

Alternative metods using animals

- Changed test of toxicity of xenobiotics: reduction of number of animals, health setback is evidence toxicity and test is terminated.
- Eye irritability is not examined when the substance irritates the skin or when the substance is a potent acid or alkali (in such cases eye irritability is expected)

THE END