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PATHOPHYSIOLOGY

Laboratory Exercises

Pavel Sobotka et al.

Pathophysiology
Laboratory Exercises

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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.

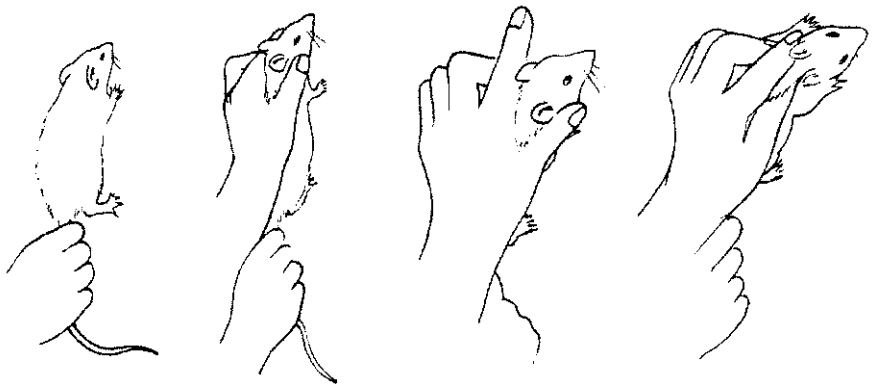


Fig. 1.2.1 Holding the rat with two hands

Fig. 1.2.2 Holding the rat with one hand

The following demonstrates an example of a laboratory protocol of an experiment.

- Name of experiment
- Report number x, date

l. Introduction: the aim of the laboratory experiment (brief description of the character of the experiment)
2. Experimental subject: kind, age, weight, sex, anamnesis (if healthy or after any surgical performance).
3. Experimental Procedures:
   a) preparation, anesthesia
   b) course of the experiment
4. Results: description of the single observation or numeral evaluation of the results obtained, e.g. including statistical treatment.
5. Conclusions: generalisation of the observations obtained in the correlation to theoretical assumptions and knowledge.

1.3 Anesthesia

Principles of anesthesia

Anesthesia can be reversible (usually) or permanent (for example in tumour pain); it causes loss of perception of all stimuli (analgesia – loss of painful stimuli only).

Basic division:
1) general anesthesia
2) local anesthesia

1.3.1 General anesthesia

Includes typically: analgesia (loss of pain perception), amnesia, loss of conciousness, inhibition of sensory and autonomic reflexes and, when necessary, skeletal muscle relaxation (using various tranquilisers and myorelaxants).

Types of general anesthesia depend on the way of administration – usually given by inhalation or intravenously, intramuscularly, rectal infusion (in children) and in small experimental animals also intraperitoneally.

Inhaled anesthetics: drugs are usually used in combination (nitrous oxide, halothane, isoflurane)

Intravenous anesthetics: mostly used for induction to anesthesia (weak or no analgetic effects), rarely for its maintenance (barbiturates, benzodiazepines, ketamine).

Signs and stages (traditionally described from observations of the effects of diethylether, Guedel's signs):

I. Stage of analgesia

- from the initial administration to the loss of consciousness; signs are: normally responsive pupils, later mild dilation, tachycardia, tachypnoe, unchanged skin reflexes.
  In this stage some short-time operations can be performed (painfull re-bandage)

II. Stage of excitement (excitation)

- during this stage (from the loss of consciousness to the beginning of the automatic respiration), some typical signs as extremely marked excitation and motor agitation, hypersalivation, increased emetic

reflex are present. In some drugs other signs (arrhythmia, circulatory instability or irregular respiration) are present.

No action is allowed during this stage. Rapidly acting drugs are used to minimize duration of this stage and reach stage 3 as fast as possible.

III. Stage of surgical anesthesia

- Signs are: automatic respiration, absent eye-lid and corneal reflex, absent reaction to pain, rhythmical eye-balls movements, sometimes nystagmus.
  In this stage most of operations (including tracheal intubation) are performed.

IV. Stage of paralysis (medullary depression)

- This stage includes severe depression of the vasomotor center in the medulla as well as respiratory center; without full circulatory and respiratory support it is lethal.
  Warning signs: maximally dilated pupils, fading fotoreaction, irregular heart action, urinary and fecal incontinence.

Neuroleptanalgesia – a special form; it is the combination of intravenously applied analgetics with neuroleptics (causes the dissociation of sensation).

Purpose of praemedication

Administration of drugs with other effects.

Importance: satisfactory rest at night before operation (praepraemedication), calm down, basal analgesia, suppression of readiness to allergic reactions, suppression of vegetative reflexes (bradycardia, hypersalivation, bronchial hypersecretion).

Examples: sedatives, hypnotics, anxiolytics, parasympatolytics (atropin), antihistaminics; commonly used combination: tranquilizers + opiats + vagolytics.

Skeletal muscle relaxants

Drugs which

A) interfere with transmission at the neuromuscular ending and suppress CNS activity (= neuromuscular blockers) or
B) reduce spasticity (increased muscle tone) – spasmolytics

Neuromuscular blockers are used primarily as adjuncts to general anesthesia.

Basic classification:

1) depolarising muscle relaxants
   Principle of action: stimulation of cholinergic receptor leads to the generation of action potential (AP) (fasciculations); antagonization is not possible.
   Example: Suxametonium (succinylcholine)
2) non-depolarising muscle relaxants
   Principle of action: competitive block of cholinergic receptors without generation of AP;
   these drugs are known as curariform medicaments. Example: Pancuronium, Atracurium;
   antagonist: neostigmin (decurarization).

Risks of anesthesia

- apnoic pause (anestetics depress respiratory centre; myorelaxants relax sceletal muscles including respiration – aspiration, anafylaxis, embolisation)
- haemorrhage, acute IM, malignant hyperthermia, hyperkalemia in myopaties, hypoventilation

1.3.2 Local anesthesia

Mechanism of the effect of LA

Blockade of the inner orifice of the sodium channel negatively influences the depolarization of nervous fibres.

The non-ionized form of anesthetic enables its penetration through connective tissue, myelin sheath and cell membrane. After intracellular ionization it blocks the sodium channel.

Ratio of the ionized versus non-ionized form depends on pH of the tissue.

Healthy tissue is slightly alkaline and therefore the anesthetic is mainly in the non-ionized form and so it easily penetrates into the cell. It leads to rapid onset of anesthesia.

In the tissue affected by inflammation pH decreases and the smaller portion of non-ionized form of anesthetic leads to its poor penetration into the cell and to a weak effect.

To reduce the absorption of anesthetic and to prolong its effect it is necessary to add vasoconstrictive agents (adrenaline/epinephrine).

It also decreases toxicity and bleeding.

Local anesthesia therefore reversibly blocks AP conduction along nerve axons and other excitable membranes (sodium channels as primary place of AP generation).

Contrary to general anesthesia, a consciousness is preserved, it does not influence breathing and communication with the patient is possible.

Places of local anesthetics effect are: spinal roots, nerve plexi and peripheral nerves.

Types of local anesthesia:

1) topic (surface, mucosal)
2) infiltrative
3) conduct
4) spinal
   - epidural
   - subarachnoid

Topic (surface, mucosal) anesthesia

Drug (aerosol) is administered on the mucous surface; used in ORL, ophthalmology (conjunctiva or cornea), in urology for anesthesia of the mucosa of the urinary tract (before an urethral catheterization).

Infiltrative anesthesia

Drug is administered by injection into the area of the nerve fibers to be blocked (zone of operation).

Conductive anesthesia

Drug is administered purposefully near nerve or nerve plexus; it leads to the anesthesia of all parts controlled by the particular nerve.

Examples of usage: conductive LA of peripheral nerves (n. radialis, medianus, ulnaris, femoralis, ischiadicus), 2nd or 3rd branch of trigeminal nerve (in stomatology).

Epidural anesthesia

The drug is administered into the epidural space; leads to a block of impulse conduction in nerve exit from the dural sac.

All kinds of nerves are affected (sensitive, sympathetic, motor).

Subarachnoid anesthesia (spinal, intrathecal)

Drug is administered subarachnoidally into the cerebrospinal fluid. We can use either isobaric anesthetics (they stay where they are applied or minimally difuse) or hyperbaric anesthetics (spread

in dependence on gravity, thus range of anesthetized zone can be influenced by positioning of the patient).

This type of anesthesia reveals a risk of severe complications (paralysis of the respiratory centre).

Most commonly used local anesthetics:

- amino-esters (Procaine, Tetracaine); they are less stable, the effect has shorter duration, but allergic reactions are more often
- amino-amides (Trimecaine – Mesokain, prilocaine); they are more stable, the effect is prolonged with less allergic reactions.

1.4 Injection technique

Proficiency in the injection technique is a necessity arising from its extensive and versatile clinical use.

- Material: syringes, needles, infusion bottles, cannulas, catheters.
- Purpose: fluid removal, medicament application, diagnostics (contrast substances).
- Application: medicaments, anesthetics, solutions, alimentation, blood and blood derivates, diagnostic solution, vaccination.
- Methods of application: intradermal, subcutaneous, intramuscular, intravenous, intraarterial (oncology), intracardial, intraperitoneal, epidural, into various body cavities.
- Fundamentals: asepsis, elimination of air bubbles, aspiration (compartment control), speed of application (irritating solutions, Ca), right indication of appropriate technique (contraindication of oil inj. s. c.), anticoagulants in the syringe if blood sample is taken.

In experimental animals the intramuscular injection is administered into the gluteal muscle of drawn hind leg.

Intraperitoneal injection replaces difficult intravenous injection in laboratory rodents (for fast absorption of substances). To avoid the damage of abdominal organs, the animal is held with the head down and we inject at an angle of about 45°. We must feel the overcome of two barriers – skin and peritoneum.

Intravenous injection in rodents is applied rarely for its difficulty. In rats there are available lateral veins on the tail, in rabbits the marginal vein on the ear (in the middle runs the artery).

1.5 Basic surgical instruments and sewing material

Instruments used for the operation on laboratory animals are identical to those used in human surgery.

Scalpels of various sizes and shapes. Scalpels with disposable blades are advantageous. For delicate incisions the scalpel is held as a pen. (Fig. 1.5.1). If a greater force is needed, the scalpel is held in the palm and pressed down with the forefinger.

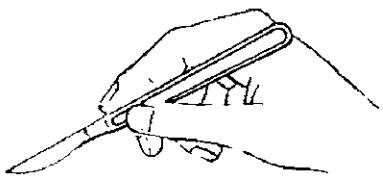
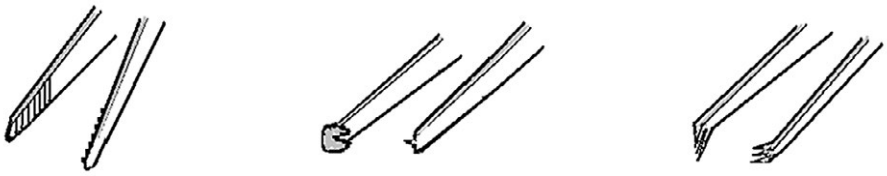


Fig.1.5.1

Scissors are straight, bent at an angle or flat. For delicate incisions ocular scissors are used. Also incision of the skin in small laboratory animals can be alternatively performed with scissors, since the skin is shifted against the base when scalpel is used.

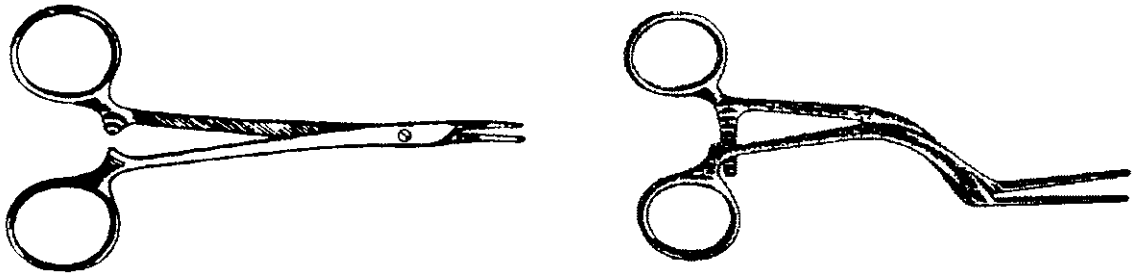
Tweezers (pincettes) are anatomical (a), surgical (b) with two hooks on one branch and with one hook on the other branch and adapting (c) with several hooks on each branch (Fig. 1.5.2).



a) **b)** **c)**

Fig. 1.5.2

Vascular forceps according to Pean (Fig. 1.5.3 a) and **Höpfner** (Fig.1.5.3. b) with a lock are used for vessels (stop bleeding). Branches ends as anatomical tweezers.



a) **b)**

Fig. 1.5.3

Forceps according to Kocher are similar to Pean (terminated with hooks as the surgical tweezers). They are used for firm grasping. (Fig. 1.5.4)

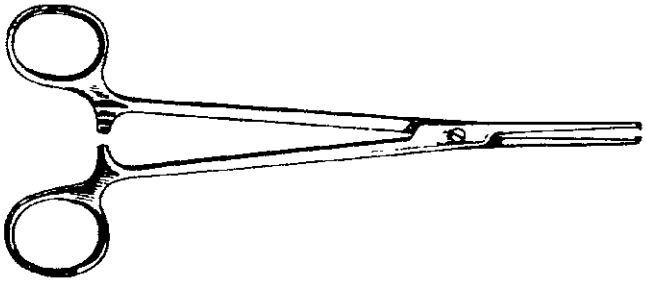


Fig. 1.5.4

Towel clamp according to Backhaus with a lock on one side and with sharp tips on the other side (Fig. 1.5.5)

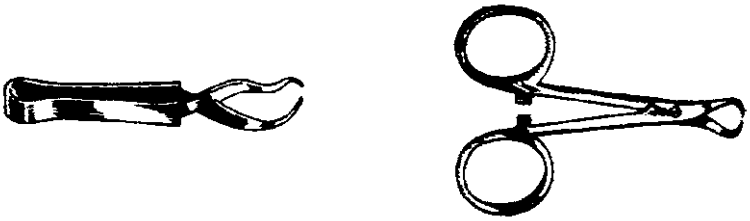


Fig. 1.5.5

Forceps for tampons (tampon holder) for disinfection of the operation field, manipulation with towels etc.

Intestinal clip with long elastic branches which can be covered with plastic material for carefully closing the intestine (e.g. by resection) (Fig. 1.5.6).

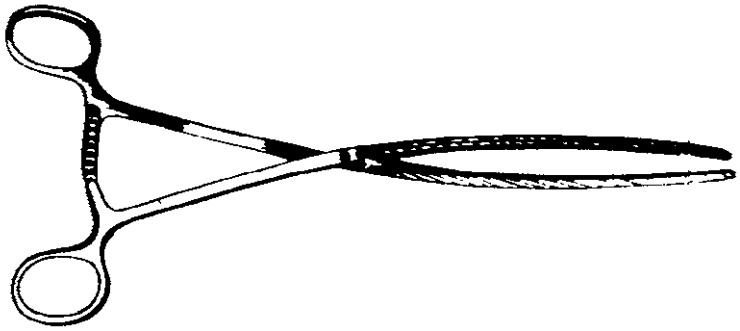


Fig. 1.5.6

Probe used for checking various cavities, channels (gland ducts) etc.

Hooks of various shape and size for retraction of operation wound. Sharp or blunt (Fig. 1.5.7, 1.5.8, 1.5.9, 1.5.10).

Fig. 1.5.7

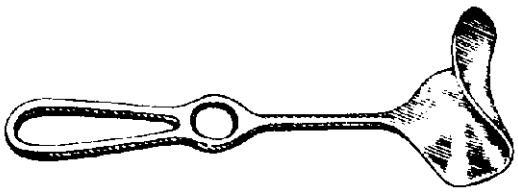


Fig. 1.5.8

Fig. 1.5.9

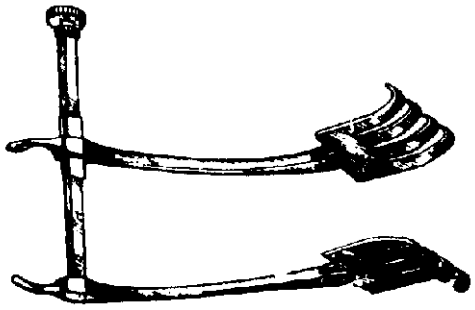


Fig. 1.5.10 ecarter (automatic hook)

Chisel spoon with sharp edges for evacuation of various material (e.g. granulations) (Fig. 1.5.11).

Fig. 1.5.11

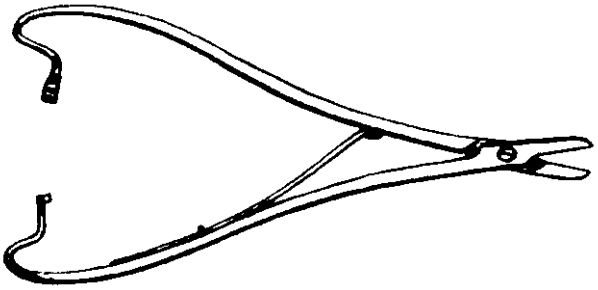


Fig.1.5.12

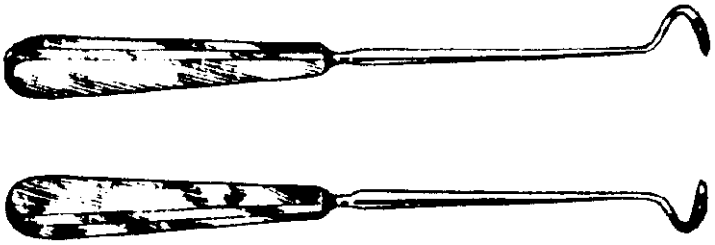


Fig.1.5.13

Needle holder of various size and type (mostly with lock) (Fig. 1.5.12).

Needles for ligation according to Deschamps (Fig. 1.5.13).

Metal hammer, **chisel** (strait, grooved) (Fig. 1.5.14), **raspatory** (elimination, scraping of periost) (Fig. 1.5.15).

Cutting pliers (forceps) for bones according to **Luer** (Fig. 1.5.16), according to **Liston** (Fig. 1.5.17).

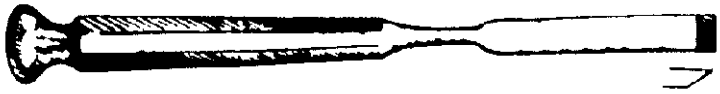


Fig. 1.5.14 Chisel

Fig. 1.5.15 Raspatory

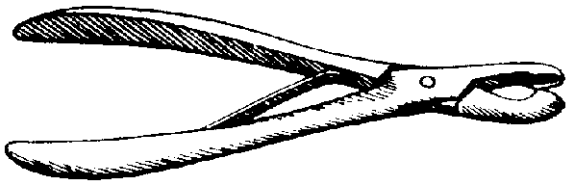


Fig. 1.5.16 Luer

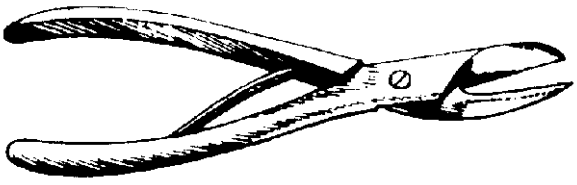


Fig. 1.5.17 Liston

Saw according to Gigli

Surgical needles are straight or more or less accurately curved. Classic needles have an eye which is threaded, atraumatic needles have the thread molded directly into the needle.

Skin needles have triangular cross-section, sharp in tip part, which cuts the tissue well. Muscle needles have round cross-section.

Sewing material

- natural (silk, catgut)
- artificial (silon, orsilon, tervalon, vicryl, monolac, chirlac)

- non-absorbable (silk, nylon, orsilon, tervalon)
- absorbable (vicryl, monolac, chirlac, catgut)

- simple (monofilament)
- braided from multiple threads (polyfilament)

1.6 Surgical technique in laboratory animals

1.6.1 General principles

- Aseptic conditions (surgery washing, sterile clothing, gloves, instruments, germicidal lamps)
- Application of general anesthesia (monitoring of reflexes – determining the surgical stage)
- Preparation of the operation field (depilation, disinfection)
- Fixation of the animal to the operating table
- Covering of the operating field with towels
- Re-disinfection
- Operation
- Closure of the wound – sewing

1.6.2 Surgical sutures

Introduction: Needles with triangular cross-section are used for the suture of firm, solid tissue. Needles with circular cross-section are used for the suture of muscles, parenchymatous organs and various membranes. The needles are provided with special eyelets which enable rapid threading.

The needle is fixed in a needle holder closer to the eye. The surgeon holds the needle holder in his dominant hand, and the tweezers in his other hand. The tweezers hold the edge of the wound and help to hold the needle when its repeated grasping is necessary. Performance of various types of sutures and surgical knots is depicted in the figures below (fig. 1.6.1–5, fig. 1.6.6, 7). The sewing of the skin is usually performed with single sutures. The needle is pulled out in direction of its curvature.

Aim of the study: Training of the surgical technique

1. Surgical knot (Fig. 1.6.1, 2)
2. Stringing of a surgical needle
3. Sewing of sutures: a) single (Fig. 1.6.3)
   b) serial (Fig. 1.6.4)
   c) pull through (Fig. 1.6.5)
   d) mattress (Fig. 1.6.6)
   e) tobacco (Fig. 1.6.7)

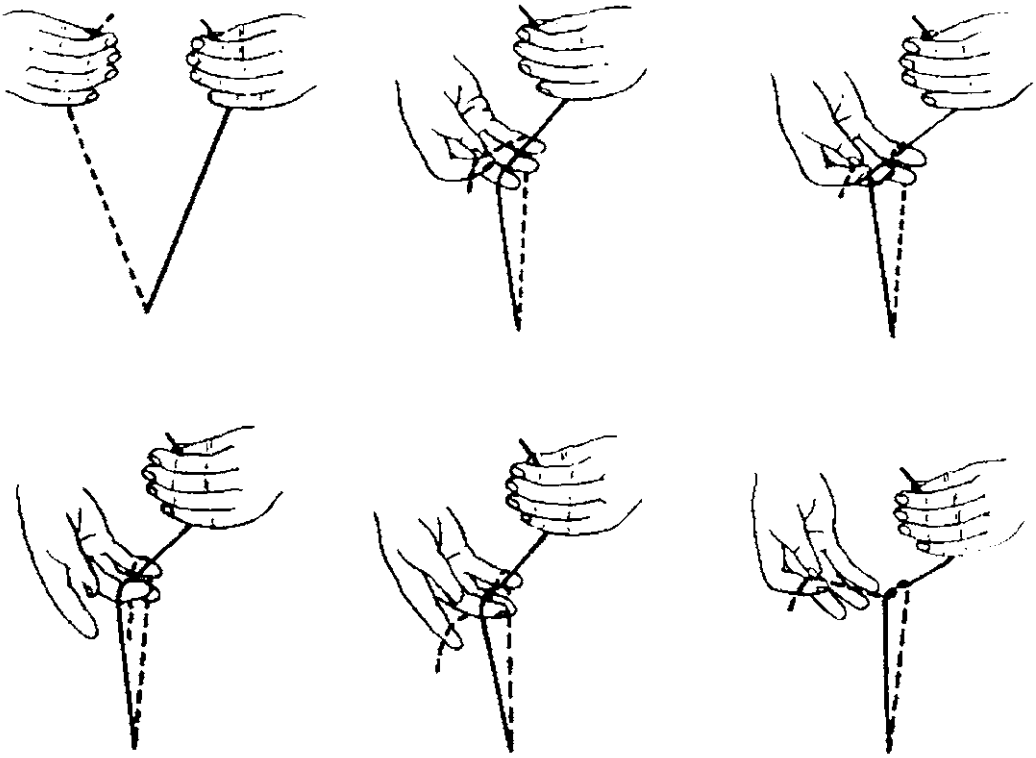


Fig. 1.6.1 Hand knotting

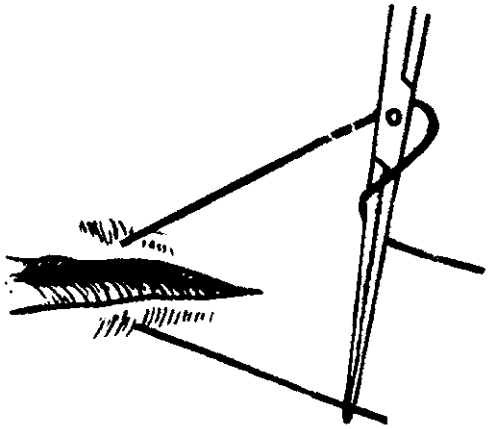


Fig. 1.6.2 Knotting using surgical instrument

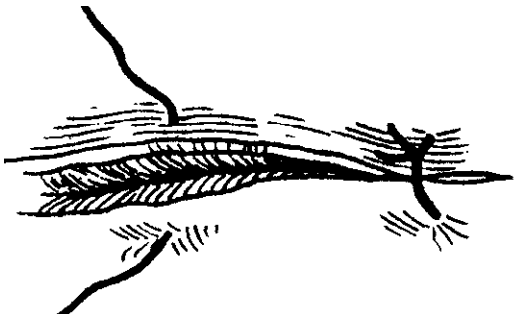


Fig.1.6.3 Single suture

Fig.1.6.4 Serial suture

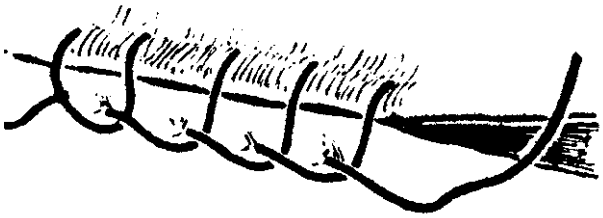


Fig.1.6.5 Pull through suture

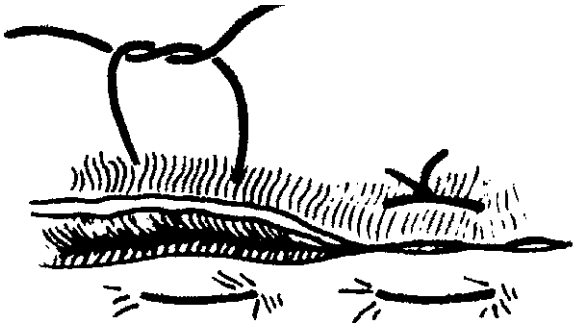


Fig. 1.6.6 Mattress suture

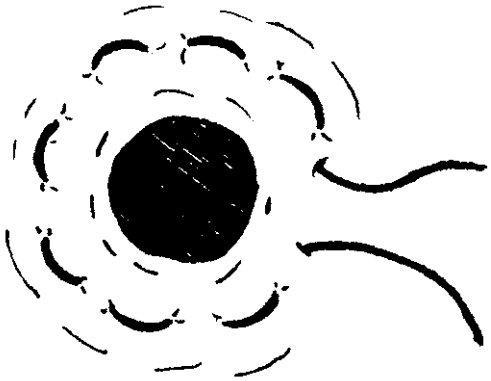


Fig.1.6.7 Tobacco suture

Adaptation of the wound – the wound edges are held together using adapting tweezers (performed by the assistant).

Removal of stitches (sutures) – place is disinfected. The free end of the suture is elevated and the suture is cut next to the skin (mucous membrane) and pulled out. By this way the sewing channel is not contaminated.

1.6.3 Cannulation of the vessels

Introduction: Vascular cannulas (or catheters) are used for repeated blood sampling or intravenous drug administration. We can use glass, metal or plastic (teflon, silon, superacryl) cannulas. Their size depends on the vessel diameter (Fig. 1.6.8 b). The arterial cannula for direct blood pressure registration consists of three arms. The arm with a narrowing (isthmus) for ligation and blunt tip is inserted into the artery. The second arm is connected to the registration unit and the third one (usually vertical) is used for filling and cleaning of the cannula (Fig. 1.6.8 a).

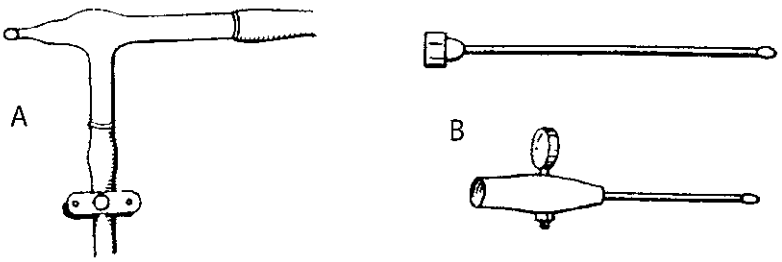


Fig. 1.6.8 a) Arterial cannula b) Two types of venous cannulas

The metal venous cannula has an olive-shaped end, facilitating right fixation in the vein. The mandrel (usually very thin wire) must have an adequate diameter and length, so that the blood in the cannula will not coagulate or escape. In our experiments the femoral artery and vein will be most frequently used.

Aim of the study: Insertion of vascular cannulas

Subject: Rat, rabbit

Equipment: Vascular cannulas of various sizes, scalpels, scissors, vascular forceps, tweezers, tampons, sewing material, pentobarbital, Mesocain.

Procedures: In an anesthetised experimental animal an incision of depilated and disinfected skin over the course of appropriate vessel is made. The muscles are bluntly dissected, the vessel released and two ligatures prepared. If we insert the cannula into the artery, its central part (towards the heart) has to be closed with pean. In the case of the vein, the peripheral part will be closed. Then we cut the vessel with small ocular scissors transversally about 1/3 of its wall, introduce the cannula and tighten the ligatures.

1.6.4 Tracheostomia (Insertion of the tracheal cannula)

Introduction: The tracheal cannula, usually glass of Y-shape or T-shape, is used for respiratory registration in anesthetized animals or for connection to artificial ventilation (respiratory pump).

Aim of the study: Introduction of the tracheal cannula

Subject: Rat

Equipment: Tracheal cannulas of various size, scalpels, scissors, tweezers, sewing material, tampons, respiratory pump

Procedures: The rat is anesthetized and fixed in the supine position. We depilate and disinfect the operation field. When we determine, that the animal is in surgical stage, we perform skin incision on the neck in the midline with a scalpel or scissors. Due to high sensitivity of this area, local anesthesia can be applied before the incision. Thereafter we continue carefully through the fascias and muscles in the midline. We bluntly isolate the trachea from surrounding connective tissue. Then we put a double ligature (one is reserve) under the trachea and base the knot on the inferior thread. With scissors we perform the T-shaped cut: transversally between the third and fourth cartilage ring and longitudinally across two upper rings. The trachea has to be slightly elevated to introduce the cannula easily. We tighten the prepared knot and fix it around an arm of the cannula. The wound is closed with several single sutures in the skin only.

Evaluation of the operation: describe potential complication (bleeding, asfyxia due to aspiration of the blood, apnoic pause after barbiturate, cardiac arrest due to inappropriate preparation (dissection near the n. vagus) and their solving (stop bleeding, aspiration of the blood from cannula, resuscitation). Finally, indicate whether the animal survived.

1.7 Basic evaluation of measured data

Introduction: Statistics is a science processing multiple data to describe the data set (descriptive statistics) or to test hypothesis and form conclusions (inductive statistics). Statistical values called also

statistics describe the statistical set of data. They are cardinal and sample. Cardinal statistics describe a cardinal set (population). It means a set containing all existing elements (individuals, measures) which fulfil conditions for involvement into the set. Sample statistics describe a sample set. It involves only a subset of all existing elements. Practically we often use sample sets, because usually it is not possible to make the measurements in the complete population. Sample statistics serve then as an estimation of cardinal statistics. To obtain exact and reliable estimation, the sample has to be large enough and representative.

The fundamental statistical measures describing the set are:

Size of the data set (*n*): number of samples involved in the set

Mean ($\bar{x}$): the arithmetic average of a set of values

Median: the middle value if samples are arranged in order of magnitude (for an odd number of samples) or the average of the two middle values (for even number of observations)

Mode: the value in frequency distribution that occurs most often

Variance (s^2, σ^2): total sum of the squared deviations from the mean, divided by number of samples (n), in the case of sample set divided by ($n - 1$)

Standard deviation (s, σ): square root of variance

Standard error of the mean: standard deviation divided by the square root of n

Experimental and clinical studies usually operate with two or more sets of data, which are compared (e.g. new medicament x control). To assess, whether differences between the sets are linked to studied factor or whether they are only randomly caused by the variability of the set, it is necessary to ascertain statistical significance of the difference. To decide, whether the differences are significant, **hypothesis testing** is used. There are many tests with various conditions for their use. The tests are divided to parametric and nonparametric ones. Basic condition required for parametric tests is normal distribution of data in all compared sets (distribution curve is the Gaussian curve). If this condition is not completed, nonparametric tests have to be used. T-test is an example of a parametric test. Mann-Whitney test is an often used nonparametric test. If couples of data are compared, paired tests are used. The examples are paired t-test (parametric), sign test and Wilcoxon matched pair test (nonparametric).

Hypothesis testing starts with zero hypothesis formulation. It is usually negative (the sets do not differ, the differences are statistically insignificant). With the tests we find the level of significance (p), it means the probability that zero hypothesis rejection is incorrect. Lower p value indicates higher statistical significance of the differences between the groups. There is common appointment that the differences are statistically significant, if $p < 0.05$.

Aim of the study:

1. Count arithmetic mean, standard deviation, standard error of the mean, mode and median.
2. Using t-test set statistical significance of differences between two different groups of measured data.

Subject: Two groups of laboratory rats, one group of males, second females

Equipment: Balance, pocket scientific calculator

Procedures: Sign number of rat males as n_1, number of females n_2. Measure body weight of all individuals. Count statistical values for the group of males and group of females. Find the statistical significance of differences of mean body weight of the groups using t-test.

Formula for arithmetic mean: $\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$ or: $\bar{x} = \frac{x_1 + x_2 + \ldots x_n}{n}$