UNIVERSITY OF DEBRECEN
FACULTY OF MEDICINE
MEDICAL SCHOOL
DEPARTMENT OF PHYSIOLOGY

A LABORATORY GUIDE FOR PHYSIOLOGICAL PRACTICES

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Authors:

Tamás Bányász, M.D., Ph.D.
Tamás Bíró, M.D., PhD., D.Sc.
Julianna Cseri, M.D., Ph.D.
László Csernoch, Ph.D., D.Sc.
János Fodor, Ph.D.
Balázs Horváth, M.D., Ph.D.
István Jóna, Ph.D., D.Sc.
Balázs Lukács, Ph.D.
János Magyar, M.D., Ph.D., D.Sc.
Péter Nánási, M.D., Ph.D., D.Sc.
Balázs Pál, M.D., Ph.D.
Zoltán Rusznák, M.D., Ph.D.
Péter Szentesi, Ph.D.
Gyula Szigeti, M.D., Ph.D.
Géza Szűcs, M.D., Ph.D., D.Sc.

Language editors:

Zoltán Rusznák, M.D., Ph.D.
Attila Gábor Szöllősi, M.D., Ph.D.

Editor:

Balázs Horváth, M.D., Ph.D.

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PREFACE

In the international practice of medical training there are - depending on the given conditions - a number of essential differences; nevertheless, some basic principles are similarly valid everywhere. One of the similarities is that physiology is considered as one of the basic disciplines to be mastered by all students of medicine. In addition, one of the most important tasks of physiology as a subject is to lay down the foundations of medical thinking in students. It follows from the character of the discipline that a practical approach is indispensable to acquire this medical thinking, i.e. through simple physiological experiments.

Another objective of practical training in physiology is to teach students the simple laboratory techniques to be used later in their professional work. Inclusions of several up-to-date and conventional human diagnostic methods were designed to serve these purposes.

From all this it follows that practical laboratory work is an organic constituent of instruction in physiology, which can be properly done, very naturally, only on the basis of suitable guidelines provided by this laboratory manual. Such a practical book, however, cannot be as universal as the theoretical ones are expected to be. Therefore, further detailed information given in the lectures and physiology textbooks - as theoretical backgrounds - are recommended to be incorporated into the practical work.

We are grateful to Olga Ruzsnavszky, Balázs Lukács, Balázs Pál, János Fodor, Ágnes Jenes, László Bárándi, Bence Hegyi, Ferenc Ruzsnavszky, Zoltán Palicz, Attila Oláh and Attila Gábor Szöllősi for the thorough revision of the Laboratory guide and the Excercise Book.

The authors hope that this laboratory guide completed by the exercise book may effectively help students to obtain proper practical experience in physiology. We are indebted for the critiques regarding the previous editions. Similar critical remarks will be always welcomed in the future, helping us to further improve this laboratory manual.

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Debrecen, 30th June, 2012

The authors

GENERAL INSTRUCTIONS

The aim and role of the physiological practices are complex. Depending on the character of the different topics, one or another aspect may dominate; however, there are some common features. Personal experiences obtained during laboratory practices help to understand and memorize the most important theoretical principles on a deeper level. The laboratory practices can only be effective if students begin the laboratory work adequately prepared. One must be familiar with the topic, with the experimental procedures applied and also with the theoretical background. In addition to this laboratory guide, physiological textbooks, lectures and previous studies may provide the required information. It is essential to be familiar with the tools and instruments used during the laboratory work.

Recordings obtained and produced during the practice sessions serve as important documentation. Application of drugs or changes in ionic environment or stimulation parameters must be marked on the recordings. Development of the effects has to be continuously recorded in time; recording of the starting and final situations only is not sufficient. Reversibility of an effect may be an important parameter of the process studied. Designation of recordings should be made during the experiment and not after the work. Adequate documentation requires calibration of the instruments (e.g. the amplification applied), as well.

The EXERCISE BOOK serves as a documentation of the completion of each physiological practice, the relevant pages must be verified by the tutor, and **it must be, therefore, preserved until the final exam**.

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1. HUMAN DIAGNOSTIC EXAMINATIONS

The aim of the physiological practices summarized in this chapter is to **measure** some of our own **physiological parameters** or to perform the same examination on one of our colleagues. These examinations offer the possibility to learn the basic rules of the interpersonal communication between the examined person and examiner. During other types of practices **non-physiological parameters** may also be analyzed. The significance of these investigations is the recognition of physiological or pathological characteristics without exact clinical diagnosis. The majority of these techniques are used in the clinical practice, whereas a few of them have merely didactic importance.

1.1. Function of the cardiovascular system

1.1.1. Electrocardiography (ECG), analysis of electrocardiograms

The **aim** of the present topic is to make sure that students become familiar with electrocardiograms recorded by the Einthoven's (standard bipolar) leads, that they can correctly evaluate the ECG recordings and that they can recognize the most characteristic aberrations.

Procedures: during the ECG examination the subject should lie on a couch with relaxed musculature as the activity of the skeletal muscles (even laughing, speaking, or lifting his/her head) causes electrical activity disturbing (or making impossible) the evaluation of the ECG recordings. The electrodes are attached to the four limbs close to the wrists and ankles, preferably above the bone (radius, tibia), where the disturbing effect of the skeletal muscles is the weakest.

An ECG gel or spray is provided for the present practice, which improves the electric contact between the recording electrodes and the skin of the patient. In the absence of these physiological saline solution may also be used, as the electrolytes are also excellent conductors. Note that the application of distilled water is not recommended as it lacks these electrolytes and which gives it a high resistance, resulting in low conductance connection between the skin and the electrodes. The metal surfaces of ECG electrodes should never be placed over socks or clothes.

According to international standards, the following color code is applied:

red = right arm
yellow = left arm
green = left leg
black = right leg (serves as ground to minimize the external electrical noise)

The ECG connection scheme applied in **standard bipolar limb leads**:

lead I = LA - RA
lead II = LL - RA
lead III = LL - LA

where: LA - left arm, RA - right arm, LL - left leg.

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After fixing the electrodes, turn the instrument on. Select the appropriate **paper velocity** (usually 25 mm/s), the required leads (I-II-III), press the **Filter** button (to reduce the electrical noise induced by the skeletal muscle contraction) and then start the recording (**Start**). As the vertical calibration (1 mV/cm) and the speed of the paper are known, the amplitude and duration of the various ECG waves and phenomena **can be expressed in mV and sec**, respectively. The duration of each ECG recording session should be at least 20 s.

Application of the schematic pattern, shown in Fig.**1.1.**, is suggested when evaluating the ECG records obtained.

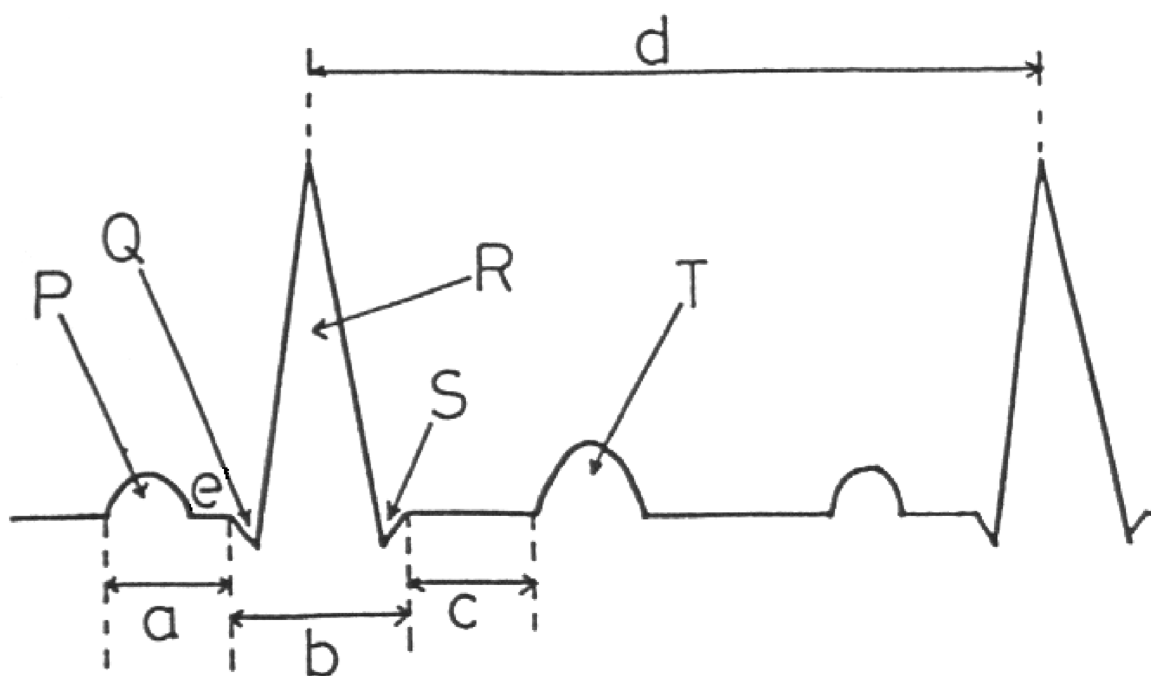

Fig.1.1.

Normal electrocardiogram obtained using bipolar lead II. The individual waves are marked by capital letters (P, Q, R, S, T). a = conduction time; b = QRS complex; c = ST segment; d = R-R distance; e = isoelectric line.

The evaluation of the ECG recording should be performed in that standard bipolar (Einthoven's) lead, where the amplitudes of the waves (most importantly the R wave) are the greatest. This is usually (but not always i.e. in the case of either right or left axis deviation) lead II.

1. The first step during the ECG evaluation is to have a look at the entire ECG recording. The examiner should look for extrasystoles and other unusual phenomena. If an extrasystole is seen, the origin and type of the extrasystole should also be noted (supraventricular or ventricular extrasystole).
2. Study the **rhythmicity** of the heartbeats (by considering the separation between the adjacent R waves).
3. Calculate the **heart rate**. If the heart activity is rhythmic, this can be assessed by determining the distance between two neighboring R waves. If the paper velocity is

25 mm/s, then 1 mm on the paper (the distance between two small dots) corresponds to 1/25 s (0.04 s). By knowing the distance between two adjacent R waves, the time between two R waves (so the length of a complete cardiac cycle) can be easily determined (the distance measured in mm X 0.04 s). As one knows the duration of one cardiac cycle the heart rate can be determined as:

HR = 60 / the duration of the cardiac cycle in seconds

However, if the heart activity is not rhythmic this technique cannot be used due to the irregularity between the R-R periods. In this case the number of R waves must be determined in a 30 s long recording period, and this number should be doubled. Note that the heart rate is still considered rhythmic if there are only small differences in the R-R distances that coincide with the breathing (respiratory arrhythmia).

4. After determining the heart rate, the rhythm generating center must be determined. Naturally, in a healthy individual this is the sinoatrial node, indicated by the fact that each individual R wave is preceded by a regular **P wave**.

5. The next step of the evaluation is the description of the individual waves. First the **amplitude** (it can be easily calculated as 1 cm=1 mV)**, duration** (on the basis of the paper velocity; see above) **and polarity** (in the case of upward and downward deflection the polarity is positive and negative, respectively) **of the P wave** should be determined.

6. Calculate the length of **conduction time** (time elapsed from the onset of the P wave to the beginning of the Q wave, marked as “a” on Fig. **1.1**).

7. Examine the shape of the **QRS complex** in each lead (marked as “b” on Fig. **1.1**). The shape of the QRS complex is mostly influenced by the sequence of depolarization within the ventricular muscle (but the QRS complex is obviously different between leads). By definition, the Q wave is the first downward deflection after the P wave. The R wave is the first upward deflection after the P wave, and the S wave is the downward deflection after the R wave. It must also be noted whether each component of the QRS complex is present. (Components of the complex may be missing in any or all of the leads, this does not necessarily indicate pathologies.) The **amplitude of the R waves** should also be determined.

8. Study the **position of the ST segment** (from the end of the S wave until the onset of the T wave) comparing its position to that of the isoelectric line (marked as “e” on Fig. **1.1**). It may be normal (matching the line), may be situated above the isoelectric line (elevated or raised ST segment) or below the isoelectric line (depressed or lowered ST segment). Both deviations have important diagnostic significance, since they indicate ischemia (inappropriate blood perfusion of the myocardium).

9. Examine the **shape**, **polarity, duration** and **amplitude of the T wave** in each lead.

10. Finally, the position of the mean electrical axis of the heart should also be determined on the basis of the amplitudes of the individual R waves and by using the Einthoven’s triangular method: One must choose any two of the three Einthoven leads (it is recommended to select the ones with the highest R waves) and measure the amplitude of the R waves (in mV). It is important to examine the chosen two leads

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at the same time, they should belong to the same cardiac cycle. These data will provide the lengths of the individual R vectors (in mm), and their direction will point toward the positive point of the corresponding standard bipolar lead. The vectors should be drawn into the Einthoven's triangle onto the two corresponding sides of the triangle, then the R vectors should be summed using the rules of vector summation. (One has to shift one vector in such a way that it should start where the unshifted vector ended, and the summation vector [integral vector] will start at the starting point of the unshifted vector and point toward the end of the shifted one.)

In addition to the electrocardiogram recorded at rest, a moderate ECG **stress test** (physical exercise; 50 W for two minutes) should be performed (the details are found in Chapter 1.1.3). **Note that changes in the position of the ST segment evoked by the physical load must be considered as severe diagnostic signs, contraindicating further tests** (details in Chapter 1.1.3.).

1.1.2. Analysis of simulated electrocardiograms

The aim of this topic is to become familiar with the exact description of physiological parameters, to recognize and characterize the pathological signs without clinical diagnosis.

Procedures: each student has to analyze and characterize a few simulated electrocardiograms provided by the tutor. Students are expected to evaluate the recordings correctly and suggest possible reasons which may be responsible for the recognized changes (naturally, only on the basis of the knowledge at the current level of their medical studies).

1.1.3. Measurement of blood pressure at rest and after physical exercise

Procedures: to measure **arterial blood pressure** the method of **Riva-Rocci** is the generally applied procedure in the clinical practice (it is common to use the initials, RR, to denote the measured values). Wrap the cuff of the manometer on one of the upper arms of the subject and inflate it, until the pressure inside (approximately 180-200 mmHg) is higher than the expected systolic blood pressure. The pressure in the cuff is measured by a **mercury- or aneroid manometer**. Start to deflate the cuff and **determine when the blood stream in the brachial artery is re-established** (i.e. when the external pressure becomes smaller than the blood pressure, and the lumen of the blood vessel becomes available for the blood flow again). In the **auscultatory method** the re-establishment of blood circulation is determined by auscultation over the **cubital artery**, using a phonendoscope. Until the external pressure inside the cuff exceeds the value of systolic blood pressure, there is no blood flow and nothing can be heard. When it falls below the systolic blood pressure value, the blood flow starts again, but as the cross-section of the artery is still smaller than under normal conditions, the **blood flow becomes turbulent**. These factors give rise to a characteristic **sound** (Korotkoff's sound), which **persists until the external pressure drops below the value of diastolic blood pressure** and smooth (laminar) blood flow is re-established in the artery. Therefore, in practice, **the systolic blood pressure is read at the appearance of the Korotkoff's sound,**

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whereas the diastolic pressure is obtained when this sound disappears. The result is usually given in a fraction form, where the numerator is the systolic blood pressure and the denominator is the diastolic value. For example, RR = 120/80 means: determined with the Riva-Rocci method, systolic arterial blood pressure is 120, whereas the diastolic pressure is 80 mmHg.

1.1.4. Examination of the arterial pulse

Procedure: in medical practice the most common method is the palpation of the **radial pulse, but the a. tibialis postrior and the a. dorsalis pedis** are also often checked to assess the blood perfusion of the legs. To perform the exercise, place the three middle fingers of your right hand over the artery in question. Note that one should never palpate the pulse with the thumb.

When studying the radial pulse, the following **characteristics** (often called **pulse qualities**) can be determined (the corresponding Latin/Greek expressions are given in brackets).

Features of a series of pulse waves:

1. **Frequency** (number of cardiac cycles per minute). The heart rate usually is around 70/min, although considerable deviations may be seen even in perfectly healthy subjects. The heart rate may be more **frequent** (tachycardia, if the heart rate is more than 100/min) or **rare** (bradycardia, if the heart rate is less than 60/min).
2. As for the **rhythmicity** of the heart beats, the pulse may be **regular** (rhythmic) or **irregular** (arrhythmic). Note the physiological changes in the pulse due to breathing (respiratory arrhythmia).

3. If the below mentioned parameters of single pulse waves are constant and each pulse wave is uniform, the pulse is **equal**. However, if the individual pulse waves are not uniform, the pulse is regarded **unequal**.

Features of a single pulse wave:

4. The rate of rise of a pulse wave may be **rapid** (celer) or **slow** (tardus). The physiological situation is between these two qualities.
5. The **amplitude** of the pulse wave may be **normal**, **high** (altus) or **small** (parvus).
6. To examine the **compressibility of the pulse**, press the artery against the bony base with your middle finger, and judge the force necessary to stop pulsation (your finger that is more distal than the middle finger cannot palpate the pulse any more). The pulse may be compressible **with medium pressure**, easily compressible, i.e. **soft** (mollis) or **hard** (durus). From this parameter one can assess the intravascular pressure (blood pressure) and the condition of the arterial wall.

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1.1.5. Examination of heart sounds

The areas where the individual heart sounds of the valves can be heard the best are indicated in Fig. 1.2.

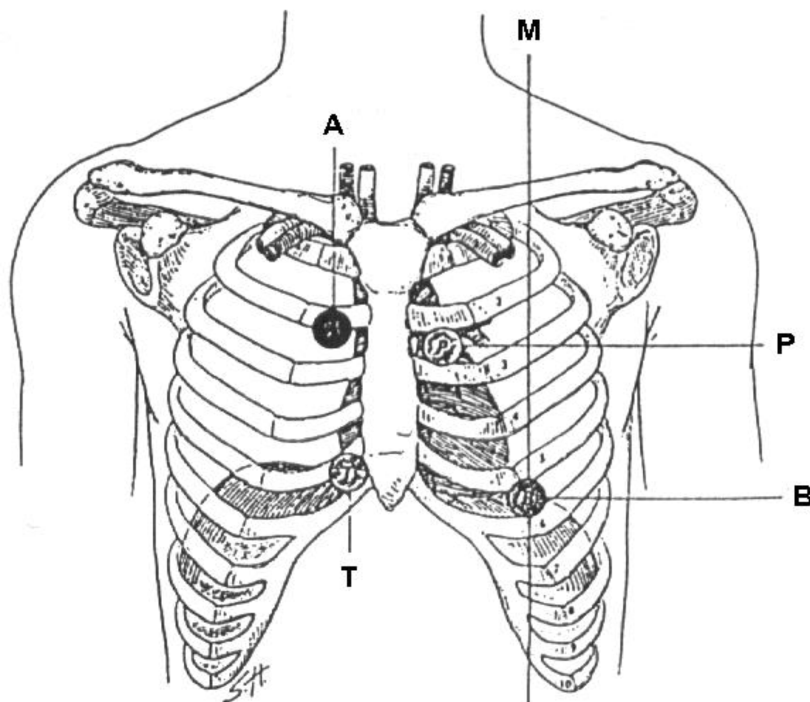

Fig.1.2.

Chest areas where heart sounds of the valves are the loudest (punctum maximum). A: aortic semilunar valve, P: pulmonary semilunar valve, B: bicuspid (mitral) valve, T: tricuspid valve, M: midclavicular line.

Aortic area: near the right sternal border, in the second intercostal space.
Pulmonic area: near the left sternal border, also in the second intercostal space.
Bicuspidal (mitral) **area**: at the apex of the heart, in the fifth left intercostal space.
Tricuspidal area: at the right sternal border, in the fourth or fifth intercostal space.

Procedures: Although under ideal conditions the heart sounds can be heard on the chest even without any instruments, certain devices are routinely applied in the clinical practice to make the life of the examiner easier. One of the possible instruments is the stethoscope, which was introduced a long time ago; nevertheless, nowadays auscultation is performed almost exclusively by means of phonendoscope. The two heart sounds can be discerned from each other with some practice: the first (systolic) sound is deeper and lasts longer, whereas the second (diastolic) one is higher, sharper and has a shorter duration.

If a murmur (pathological heart sound) is noticed one should indicate whether the sound is present during the systole (systolic murmur) or during the diastole (diastolic murmur). Characterize the intensity, duration and the direction of conduction, as well as the punctum maximum of the given pathological noise. Please note that the intensity of the murmur may change when it is present: it may become louder (crescendo) or its intensity may decrease with time (decrescendo murmur).

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1.2. Function of the respiratory system

The aim of the present exercise is to measure the most important respiratory parameters as well as to determine the metabolic rate. The changes of various pulmonary parameters will also be noted after physical exercise and in the case of simulated asthma bronchiale. Those students who are known to suffer from a respiratory disorder should not take part in the lab as a patient.

1.2.1. Determination of the basic respiratory parameters

The respiratory parameters determined in the frame of this practice fall into two major categories, as we distinguish between static and dynamic parameters. The static parameters describe the various volumes and capacities (e.g. tidal volume and inspiratory capacity), and they characterize the volume (or “size”) of the various lung compartments as well as the mechanical properties of the ribcage and the strength of the respiratory muscles. However, these static parameters provide limited information about the airway resistance. In order to assess the airway resistance, the dynamic parameters are also determined, which characterize the rate of airflow in the respiratory tract during inspiration and expiration (i.e. forced expiratory volumes, forced inspiratory volumes and peak flow rates).

The above mentioned parameters are determined using a spirometer. Spirometry is designed to determine the effectiveness of the various forces involved in the movement of the lungs and chest wall. The values obtained provide quantitative information about the presence and degree of obstruction in the airways and about the amount of air that can be inspired or expired.

Procedures

The examination is carried out using a PRE-101 type, computerized ergospirometer on Fig. **1.3.**

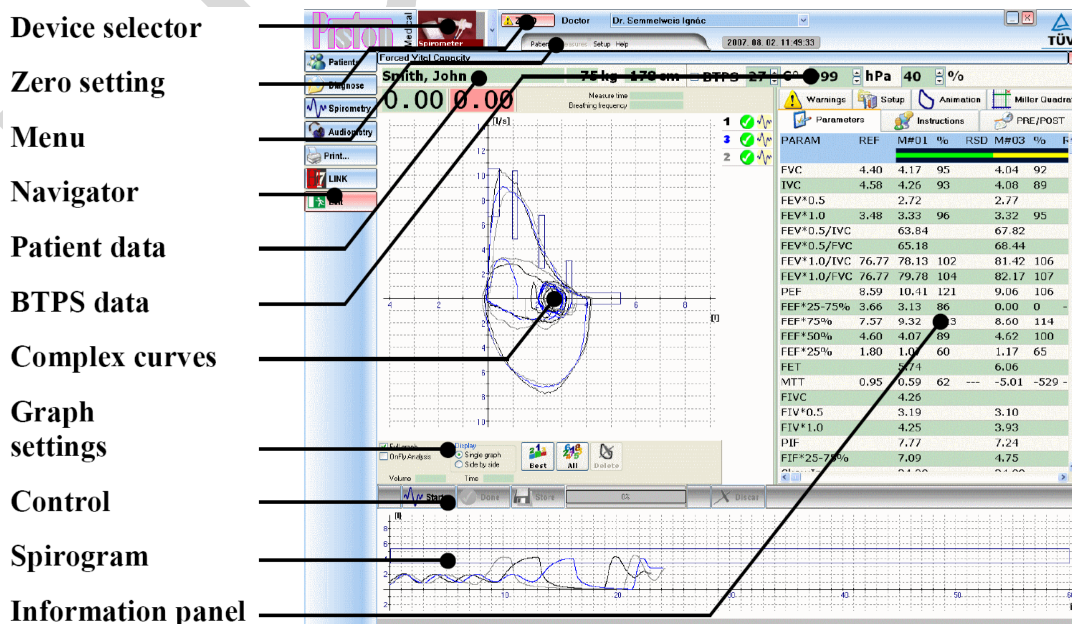

Fig. 1.3.

The measurement screen of the ergospirometer

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The important parts of this screen regarding performing the practical:

Zero setting

Runs manual Zero setting of the selected device. Without manual Zero setting the system automatically sets zero before all measurements if necessary.

Menu

The program's general main menu, which contains the grouped basic functions.

Navigator

Sidebar that switches between the basic phases of daily routine measurements.

Patient data

Contains the most important measurement data for the patient selected from the database.

BTPS data

These are the environmental data (temperature, air pressure, humidity) measured by the device.

Complex curves

The more complex curves of the individual measurement operating modes. For example, in case of FVC measurement, the flow-volume loops.

Graph settings

This is where you can set graph display modes. These settings are also available on the Options panel.

Control

This bar contains the basic control functions during the measurement. The appropriate Function buttons are shown in square brackets:

· Start measurement **[F3]**
· Start special measurement section **[F4]**
· Finish measurement after a successful measurement **[F5]**
· Stop or abort measurement (for example, in case of malfunction) **[ESC]**
· Store, print

Spirogram

Volume-time graph, which monitors the patient's breathing during the measurement.

Information panel

This section contains information, settings, functions:

· Current measurement parameter list
· Measurement related warnings, error messages
· Measurement instructions

The patient is connected to the device using a sterile mouthpiece that is attached to the pink colored flow-meter of the device. A **nose clip** is used to prevent breathing through the nose. The **changes in volume** are measured by the device with high static and dynamic sensitivity. To achieve good and accurate measurements it is necessary to **calibrate** the device which should be done by the tutor prior to the measurements, if necessary. The spirometer used on the labs is an open system, the patient breathes from the air of the examination room. In case of further questions and enquiries, a **detailed manual is available next to the spirometer.**

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Before starting the measurements, it should be confirmed that everything including the five connecting tubular parts of the pink colored flow-meter and the attached mouthpiece are completely dry. Input the patient data into the computer after clicking on the **[New Patient]** button.

Identifying data

Data essentially identifying the patient: Name, date of birth, social security number, sex, etc.

Body mass index (calculated value)

The patient's current body mass index: body weight divided by square of the height of the patient in meters.

List of incomplete fields

A list of fields that either have to be completed (indicated in pink) and are still empty, or that have been filled out incorrectly.

Control panel

Basic database operations: new patient, modification, store.

Anthropometric data

You have to enter the patient's body mass and height to calculate reference values.

New patient

To enter a new patient, press the **[New Patient]** button. Complete the fields and make sure that two patients cannot have the same identifier. To store the patient, press the **[Save]** button. You will receive feedback about the success of data storage. If you do not wish to save the data, press the **[Cancel]** button.

Modify data

Select the patient to modify then click on the **[Modify]** button. After modification press the **[Save]** button. You will receive feedback about the success of data storage. If you do not wish to save the modified data, press the **[Cancel]** button.

To preserve consistency and for future searches, it is **not possible to delete** from the database. All diagnosis have traces in the database.

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After connecting the patient apply the nose clip and start the needed measurement.

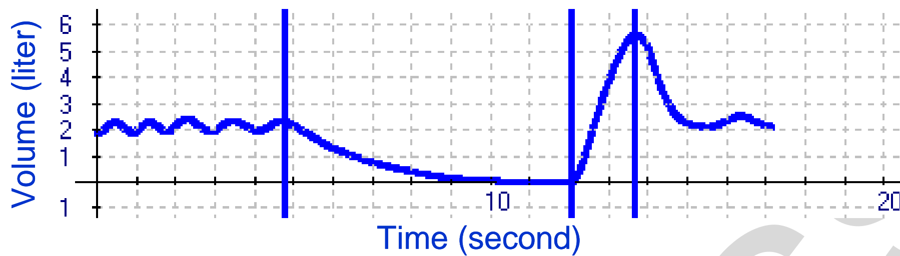
Measurement process

Instruct the patient to perform the maneuver required for the given measurement (Fig. **1.4.**), which should be completed in 60 seconds. Push the **[Done]** button to stop the measurement. Push the **[Discard]** button to delete the measurement.

To determine the **static pulmonary volumes**, after starting the **<u>IVC (Inspiratory Vitalcapacity) measurement</u>** the test should begin with at least three cycles of normal breathing. The respiratory frequency appears on the screen and a spirogram is shown. After this, the patient is requested to perform a **complete deep expiration** followed by a **complete deep inspiration**, followed by a return to normal breathing. During the one minute long measurement the device continuously shows the average of the respiratory frequency calculated from the beginning to the actual time point, but

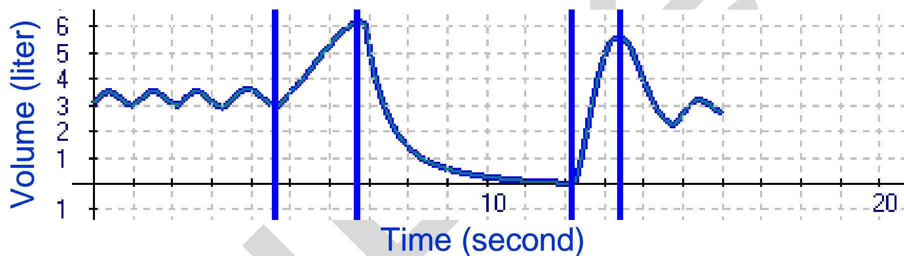
after finishing the measurement this value will not be listed, so it is advisable to write it down approaching the end of the measurement!!!

The **dynamic parameters** are determined using the **FVC (Forced Vitalcapacity) measurement**. The test should begin with at least three cycles of normal breathing. The patient should then perform a **deep inspiration**, followed by a **fast and forceful expiration**. At least 3-4 seconds should elapse between the end of the forceful expiration and the initiation of the next **fast and forceful inspiration**. Note, that during this measurement not only the volumes of air inhaled and exhaled are important, but the velocity of the air flow as well.



static

phases: normal breathing; complete deep expiration; complete deep inspiration; return to normal breathing



dynamic

Phases: normal breathing; deep inspiration; forced exhalation; forced inhalation; return to normal breathing

Fig. 1.4.

Protocols for the measurement of respiratory parameters

The Miller Quadrant (Fig. **1.5.**) available in FVC measurement Interpretation is an effective graphical tool which helps making the lung function diagnoses.

Its vertical axis shows: $FVC\ (\%) = FVC_{Actual} / FVC_{Normal} * 100$,

and its horizontal axis depicts: $FEV1\ (\%) = FEV1_{Actual} / FEV1_{Normal} * 100$,

where Actual means the value of the patient and Normal means the reference value of the patient established by standards taking into account the age, sex and antropometric parameters of the patient. The diagram is divided into four quadrants from which the likely diagnosis can be read: Normal, Restrictive, Obstructive or Combined.

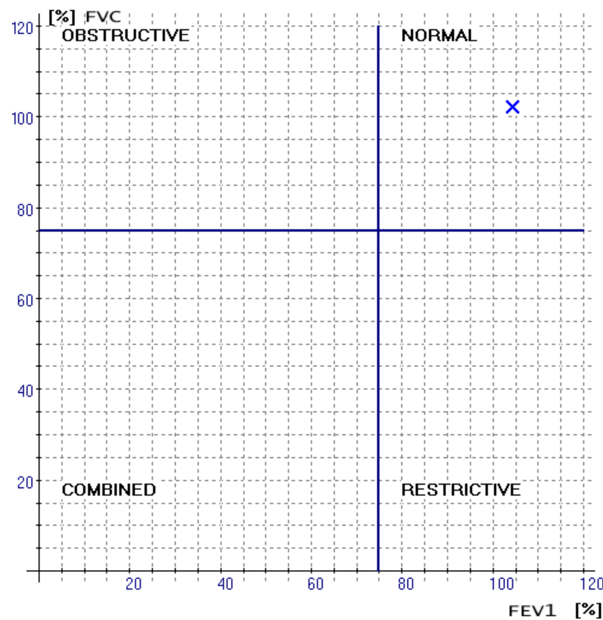


Fig. 1.5.
The so-called Miller Quadrant helps to evaluate the respiratory state of the patient

After determining the resting pulmonary parameters, moderate physical exercise should be performed, and the IVC test should be repeated again.

1.2.2. Determination of the resting oxygen consumption and the metabolic rate

Preparation: The patient should lay down on the examination bed and accommodate him(her)self in position so that (s)he should be able to stay still for at least 30 minutes. The patient can use a face mask for this measurement but a mouthpiece combined with a nose clip is also a good alternative.

Procedure: In the present measurement the O_2 consumption is determined by the spirometer in the **Ergospirometry measurement** (Fig. **1.6.**). In order to get reliable data, the person should be breathe calmly and continuously for at least 5 minutes (steady-state). Choose the **[Tidal test]** option for **[Ergometer type]** in the **[Protocol]** menu, then start the measurement by pressing **[Monitor]** button. The patient should not move at all. It takes approximately 20-30 minutes to achieve stable breathing and gas metabolism. In case of getting stable values click the **[Start]** button. The program frames the steady-state periods. The actually measured resting energy expenditure (REE) value is continuously updated. If all criteria are satisfied in the whole of a minimum 5 minute long period the program shows the message: **Steady state reached.** Click on the **[Done]** button and the measured values can be visualized by clicking on the **[Result]** button.

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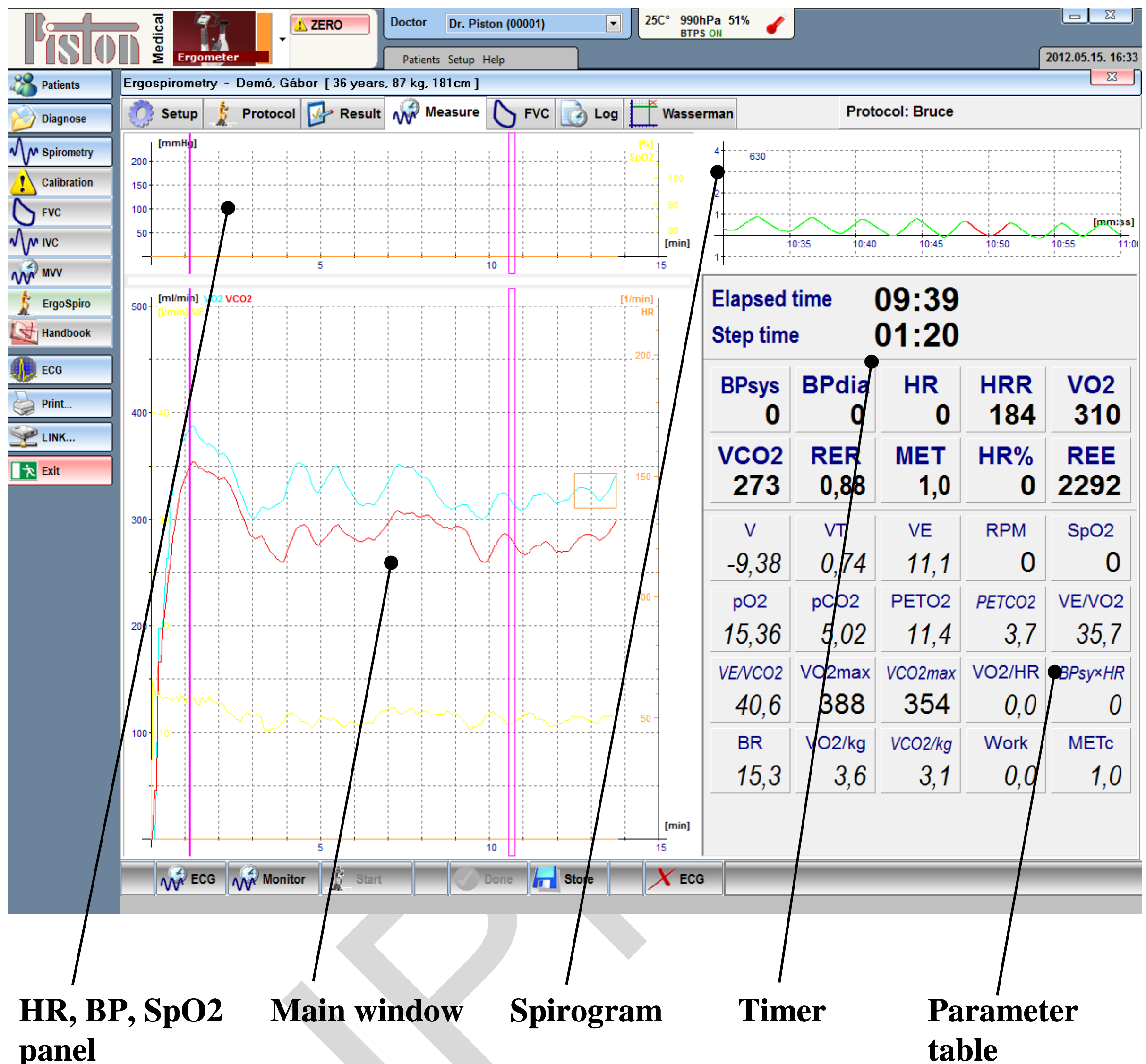

Fig. 1.6.

The screen of the ergospirometric measurement

DRAFT

HR, BP, SpO2 panel

The most important cardiologic parameters are monitored here continuously during the measurement.

Main window

The most important respiratory parameters (VO2, VCO2, VE) are monitored here continuously during the measurement.

Spirogram

To monitor tidal volume, respiratory frequency during the measurement.

Timer

To monitor the time elapsed during the measurement. A upper counter shows the whole length of the measurement, the lower depicts the length of the steady state.

Parameter table

To monitor the physiological parameters during the measurement.

To calculate the **metabolic rate** use the following equation:

MR = VO2 (L/min) x 60 x 20 kJ/L,

where VO2 means the amount of consumed O_2, 60 in order to calculate the MR for one hour and 20 kJ/L is the caloric equivalent of O_2.

Be careful as the measured value for VO2 is given in mL/min by the machine so it should first be divided by 1000!!!

The result yielded this way should then be normalized for the body surface area, which can be determined by the diagram below (Fig.**1.7.**) Connect the two points representing the body height and weight with a ruler, and read the value of the body surface area where the ruler intersects the middle axis.

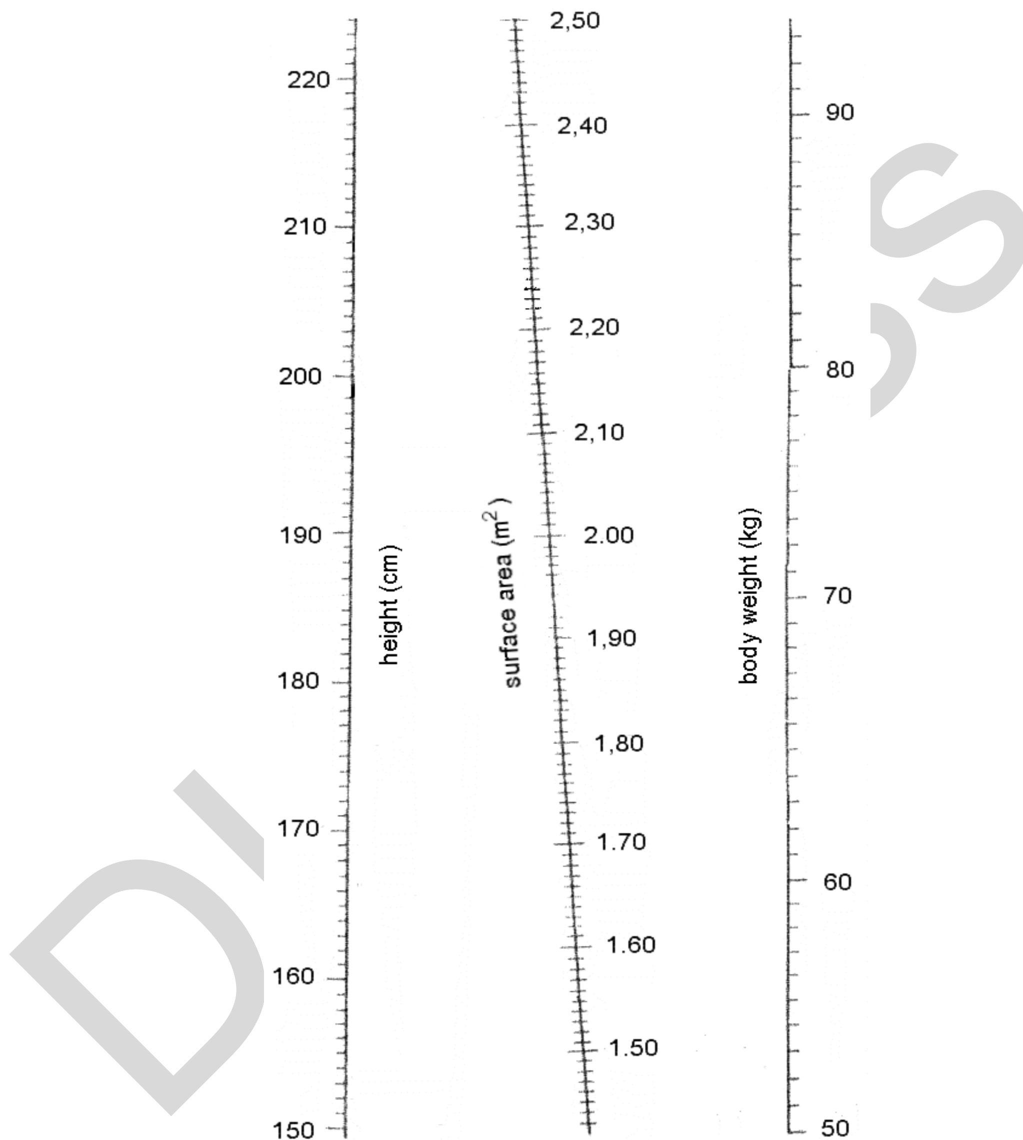

Fig.1.7.
Nomogram applicable for the determination of the body surface area.

1.2.3. Demonstration of abnormal pulmonary parameters

Asthma bronchiale (a disease characterized by highly increased airway resistance due to bronchoconstriction) can be simulated in the present setup by attaching a narrow tube to the mouthpiece. This arrangement allows the investigation of the changes of the various respiratory parameters under this pathologic condition.

1.3. Effects of physical exercise on the cardiorespiratory system, study of restitution

Tests involving physical exercise can be carried out in the clinical practice for diagnostic or prognostic purposes; they may be used to diagnose unknown diseases, or to check sportsmens' parameters.
The aim of the present practice is to investigate how various cardiorespiratory parameters change during and after physical exercise. For physical load, we use a bicycle ergometer during practice. As in the frame of these measurements changes of various parameters will be studied as the function of time following physical exercise, it is essential to organize the group to work very efficiently. It is highly advisable to share the task between the members of the group, i.e. one should be responsible for the blood pressure measurement, somebody else for the Impedance Cardiography recording, etc. During serial measurements, copying the raw data usually requires additional students' cooperation.

Abnormal responses to exercise

Please note that this measurement should only be performed on healthy individuals in good physical condition. The entire test should be taken very seriously, and if the slightest symptom of abnormal reaction is observed, the test must be immediately terminated.

Please note that **any type of angina (chest pain) and breathlessness may indicate cardiac disease, and therefore the test must be ceased.** The majority of subjects with cardiac symptoms can maintain a normal cardiac output at rest but cannot increase it sufficiently during physical exercise. The insufficient O_2 supply to the myocardium causes a decrease in the pump function of the heart, consequently, **the systolic value of blood pressure does not increase**. **In the case of severe dysfunction of the left ventricle the systolic pressure may even decrease.** A marked fall of systolic pressure early in the exercise, or a drop greater than 10 mmHg from the resting value should be regarded as indications to immediately stop the test. If the subject cannot produce a peak systolic pressure of 130 mmHg or greater during the exercise, very severe dysfunction of the left ventricle should be suspected. **An increase of the diastolic pressure exceeding value of more than 20 mmHg should be regarded as an abnormal response, too.**

The following symptoms or diseases indicate situations when the test should not be started or (if they were not present previously) must be immediately terminated:

Absolute contraindications

Unstable or crescendo angina pectoris
Severe cardiac arrhythmia
Aortic stenosis

Acute diseases such as myocarditis, pericarditis, endocarditis, infections, etc.
Asthma bronchiale

Relative contraindications
Arrhythmias, AV block, tachycardias
Pulmonary vascular hypertension
Essential hypertension
Situations that reduce exercise capacity (e.g. drugs, psychic stress, etc.)
Peripheral circulatory diseases (arterial obstruction, thrombophlebitis, etc.)

In physiological practice, the relative contraindications should be considered as absolute ones!

Those students who produced abnormalities in ECG, blood pressure, respiratory parameters or metabolism during exercise **must not be active participants of this complex physical test**.

Attention! Performance of the exercise test is allowed exclusively in the presence of the teacher. Any unpleasant subjective signs must be indicated immediately.

Absolute end points – The test must be immediately terminated in the case of the occurrence of any of the following symptoms
Progressive angina pectoris
Fall in systolic blood pressure or heart rate
Severe dyspnea, pale skin
Ventricular tachycardia (or fibrillation)
Elevation of ST segment

Relative end points – It is highly advisable not to continue the test in the presence of any of the following symptoms
Chest pain in the absence of ST segment change
Fatigue
Marked ST segment depression (5 mm or more)
Atrial arrhythmia, ventricular extrasystoles
Marked elevation of blood pressure
Attainment of predicted submaximal heart rate
Development of AV block

A person's maximal heart rate (HR_{max}) can be calculated;
HR_{max} = 220-age (in years)
A person's submaximal heart rate (HR_{submax}) can be calculated;
HR_{submax} = 200-age (in years)

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Impedance cardiography

Impedance cardiography (ICG) is a suitable method to measure stroke volume and cardiac output in a non-invasive way. Impedance is the sum of the ohmic, inductive and capacitive resistances of any conductor in the presence of alternating current (AC). ICG measures the impedance changes of the thoracal organs and tissues using high frequency (40-100 kHz) and low intensity (2-4 mA), therefore biologically indifferent current. The impedance of the chest is inversely proportional to its fluid and directly proportional to its fat content. Impedance changes occur in synchrony with the pumping function of the heart, and these changes are directly proportional to the amount of ejected blood. The ICG device monitors and detects ECG and heart sounds (phonocardiogram, PCG) as well, in order to determine the reference points of the cardiac cycle. The Kubicek formula is used to determine the stroke volume from the measured data using the following form:

$$SV = cons \times L^2 \times L\ VET \times dZ/dtmax\ /\ Z_0^2$$

where

SV	stroke volume (mL)
cons	a hematocrit dependent constant (ohm x cm; the differences in hematocrit are omitted in this practice)
L	distance between the cervical and thoracal measuring electrodes (cm)
LVET	left ventricular ejection time (sec)
dZ/dtmax	maximum of the ICG's first derivative (ohm/sec)
Z_0	basal impedance (ohm)

By modifying the original formula (taking into account various antropometric differences) the stroke volume calculated from the impedance changes correlates well with the stroke volume calculated using Fick's method (maximum ± 20 % deviation).

Useful parameters determined by the ICG device:

HR	heart rate (L/min)
SV	stroke volume (mL)
CO	cardiac output (L/min)
CI	cardiac index (L/min/m^2)
SVR	systemic vascular resistance (dyn x sec / cm^5)
S	ejection rate (ohm/sec)
QS2	time of electromechanical systole (ms)
PEP	pre-ejection period (ms)
VET	ventricular ejection time (ms) (calculation is different from that described in the book, its average value is somewhat higher)
P/V	PEP/VET

Important: If A_2/A_1 (basal curve A_{max}/derivated curve A_{min}) is higher than 0.85, the measurement is NOT valid – as indicated by the machine (blinking)!

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Placement of the electrodes:

First the white measuring, then the marked (blue dot) generator electrodes of the ICG should be placed onto the half-naked patient.

Appropriate positions of the measuring electrodes:

-first pair: symmetrically on both sides of the neck in the supraclavicular region.

-second pair: on both sides of the chest at the level of the processus xyphoideus in the middle axillary line.

The generator electrodes should be placed 5-8 cm above and below the measuring electrodes on the neck and on the chest, respectively.

The ECG electrodes should be placed on the chest according to the electrode positions of the standard bipolar leads (red: on the right shoulder at the distal part of the clavicula; yellow: on the left shoulder at the distal part of the clavicula; green: on the left side of the chest, 5 cm below the generator electrode; black: on the right side of the chest, 5 cm below the generator electrode).

The PCG microphone should be placed on the apex of the heart and it should be fixed with the elastic rubber band provided. (Note that this is a very delicate part of the apparatus, it is quite expensive and it breaks easily if it falls down; so be careful…)

The PCG-microphone should be connected to the middle socket (labeled as "PH") of the adapter.

Course of the measurement:

Switch on the ICG. The program starts automatically and operates in Hungarian only, therefore the translation of the most important menu points is given below in brackets. Keyword (kulcsszó): '111'. After pressing *Enter* several times the MAIN Menu is reached. Use the *arrows* to move up/down between the individual menu points and *Tab* to move left or right. Press *Enter* or the different colored letters to reach the various menu points.

Filling out the patient's details (Páciensadatok beírása):

Name (A páciens neve); identification code (Azonosító kód) can be anything.

Date of birth (Születési idő): year, month, day; age (Kor): automatically calculated

Sex (Nem): F=male, N=female

Diagnosis (Diagnózis): pressing *Space* brings up a list of possible diagnoses. Choose sine morbo ('healthy'), than press *Enter* and *Esc*!

Measurement identification (Mérésazonosító): this can be anything that is related to the actual conditions (eg. rest, load etc.) OR date (year, month, day) + group number.

Distance between electrodes (Elektródatávolság): the vertical distance between the planes determined by the measuring ICG electrodes on the neck and on the chest (it should be given in cm).

Weight (Testsúly) in kg; height (magasság) in cm; RR Syst. and RR Diast in mmHg.

The next three parameters can be calculated from chest X-ray only. Using these the

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machine automatically calculates the antropometrical type. As we do not have X-rays of the selected students available, these points should be skipped (by pressing *Enter*) and the anthropometrical type should be determined on the basis of the body composition of the subject ('Antropometriai alkat' point: p: short and fat; l: lean; a: athletic). When everything is ready, *Esc should be pressed.*

Monitoring (Monitorozás)

The ICG parameters can be continuously observed if this menu point is selected. Pressing *F9* swaps between the 'ECG only' (Einthoven I, II, III leads in green) and the 'everything (Mind)' function; in the latter case the *PCG (white), ICG (light blue) and ECG (green) recordings* can be simultaneously observed on the screen. Using the "1", "2" or "3" buttons the lead with the highest R wave can be selected. To record the best possible heart sounds, it may be necessary to carefully reposition the PCG-microphone. By pressing *Esc,* the MAIN Menu can be reached.

Measuring (Mérés)

1. You can set 8, 16 or 60 sec long measurements. 8 sec is usually enough; nevertheless, 16 sec should be selected in the cases of cardiac arrhythmias.
2. During physical load, the 'Sorozatmérés' (multiple measurements) menu should be selected in the following way:

Interval between measurements (Ütemezési idő): 1 min (This can be changed during exercise: *Insert* increases, *Delete* decreases the interval.)

Pressing *I*, than *N* twice and *Enter* several times accepts the previously set alarm parameters.

During the experiment the program automatically asks for the blood pressure before each measurement; and it even forewarns 30 seconds before the blood pressure determination becomes due.

Displaying the curves (Görbék megtekintése)

It is possible to zoom in and out using the + and – buttons, and scroll using the right and left *arrows*.

Processing (Feldolgozás)

Before processing, the patient's details should be entered (blood pressure, alterations of the experimental conditions). *Enter* key loads the previously measured parameters within a few seconds. Pressing *F2* shows the corrected curves (correction is needed as the ICG shows respiration-related changes, too). The red curve is the first derivative of the ICG (dZ/dt). Pressing *N* prints out a representative curve.

Pressing *Esc* twice opens the MAIN Menu. The subsequent menu point can be ignored, it has no relevance regarding the present practice.

Save the data after each measurement and processing choosing the Write to Disk (Lemezreírás) and Save (Mentés) submenu points.

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When all measurements are completed, it is possible to load all the processed data using the Load from Disk (Olvasás a lemezről) function in the MAIN Menu and pressing *Enter.* After choosing the 'measured curves (Mért görbék)' menu point, and selecting the patient using the *arrows* and *Enter,* the measurements can be initiated by pressing *Space* and *F9.* After a few seconds, the parameters show up in a table (hit *N* for the activation of the printing function).

Evaluation of the results

The obtained parameters should be evaluated in the following manner.

a. Changes in ECG

In a physiological situation, during exercise the **heart rate increases**, the **PR interval shortens**, the **P wave** becomes **higher** and there is a downward displacement of the PQ junction, therefore, the **PQ segment should be considered as the isoelectric line** (Fig.**1.8.B.**) The **amplitude** of the **QRS complex**, **R wave** and **T wave** tends to **decrease** during exercise. The normal **ST segment** during exercise is steeply upsloping, convex, and returns to baseline within 0.04-0.06 seconds (Fig.**1.8.A.**).

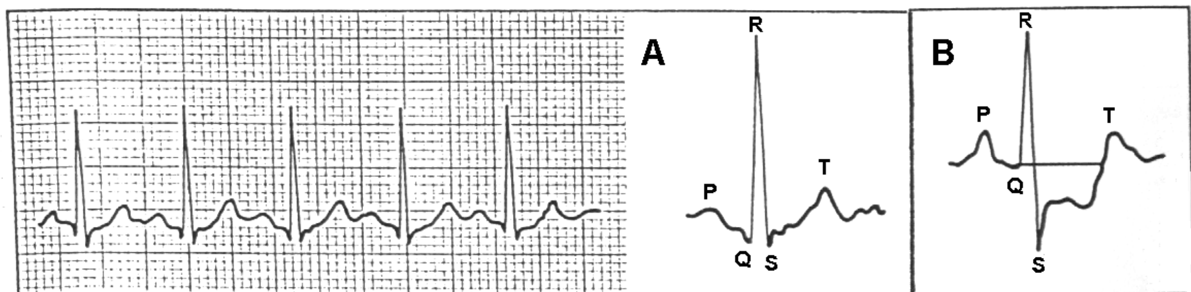

Fig.1.8.

A: Physiological ECG response to exercise.

B: Determination of isoelectric line in a pathological case.

Pay special attention to the position of the ST segment comparing its position to that of the isoelectric line. Elevation or depression of the ST segment may indicate ischemia, although ischemia is not the only reason for these changes (e.g. hyperventilation can also evoke ST depression, etc.). Abnormal responses indicating vegetative lability are shown in Fig.**1.9**.

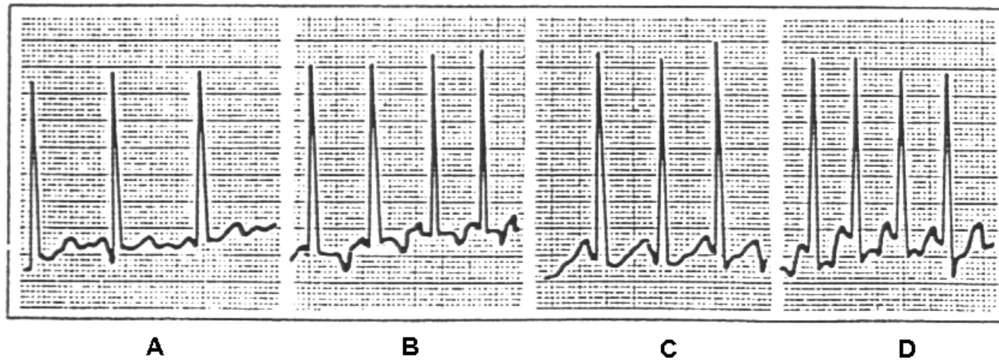

Fig.1.9.
Abnormal responses indicating vegetative lability (vasoregulatory asthenia). **A**: rest, **B**: hyperventilation, **C**: 7 minutes exercise, **D**: immediately after the exercise.

Changes of the ST segment and T wave often occur during vegetative lability. ST segment depression appears early during exercise, it often returns to normal level during maximum exercise and it may reappear in the recovery period, but the vasoregulatory asthenia itself can cause various other phenomena.

b. Changes in other parameters

In a physiological situation, during exercise the **systolic blood pressure increases**, the **diastolic blood pressure mildly decreases** or hardly changes, which leads to an **increased mean arterial blood pressure**. Both the electrical (PEP) and the mechanical (VET) **systole shortens**, and the **decrease of the diastolic period** is even more pronounced than the changes of the systole. The **cardiac output markedly increases,** the **stroke volume** also **increases,** but the actual increase very much depends on the physical fitness of the subject). The **ejection rate increases**, and under physiological conditions the **peripheral resistance decreases**.

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1.4. Examination of the nervous system and sensory organs

General considerations

The participants of the examination are the examiner and the subject, hence the lack of their effective cooperation can make even the simplest examination difficult. During the practical classes we will perform the examinations on our fellow students, who, supposedly, possess the knowledge necessary for cooperation in the examination procedures. However, we must prepare ourselves for those cases when the tests are performed on subjects who have no idea about them. Thankfully many of your fellow students as well as examiners will adequately “simulate” this state of ignorance at the time of the practice or closing lab.

Thus, we must be capable of conveying the knowledge necessary for cooperation to anyone, in a short time. We have to describe the aim of the examination and tell the subject what his role will be, what we shall do and what will be his or her task. The examination should not start until we are sure that the subject understands what he is expected to do.

Before starting the test, we must find time to ask questions. The examiner asks the subject whether he has previously noticed any change or dysfunction in the function to be tested, and how he evaluates his own performance.

During the examination we should try follow a system. The best system is the one that we can implement with the highest efficiency, while observing several basic principles for the sake of success. There are some examinations that can never follow other examinations immediately (e.g. pupillary reflex examination should never come after ophthalmoscopy since, on account of the intense light, the pupils will remain constricted for several seconds). The order of examinations given in this chapter fulfills the general requirements. The performance of the paired organs must be examined separately, since there are pathological forms that attack only one of them. The identical examinations, however, must immediately follow each other, so that the performances observed can be compared.

Let us always keep in mind the principle of ‘**nil nocere’** (Do no harm!). In the course of the examination we should avoid any stimuli and technical tricks that may be unpleasant or even noxious. There is a need for a lot of patience and endurance, not only until the necessary skills are acquired, but also thereafter, when unnecessary haste, impatience or superficiality may lead to erroneous diagnoses.

One thing is very important: we have endeavored to collect, for the descriptions, the most important sets of knowledge. All this, very naturally, is impossible to acquire merely through reading. To master the necessary skills one needs a lot of practice. Practice is just as indispensable condition of success as is the proper knowledge of the theoretical material (not described here) necessary to perform the exercises.

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1.4.1. Examination of the cranial nerves

1.4.1.1. The 1st cranial nerve

The first step in the examination, just as in other cases, is a thorough questioning of the patient to record the necessary anamnestic data. We should not forget to ask about such banal events as a running nose. One must lay emphasis on questions whether he has or has not lately observed any change in his sense of smell, whether he can sense odors in the same way as others in his environment, whether odors noted by his environment avoid his attention or he senses odors that others in his environment cannot. In performing the examination we must observe the general principles stated for paired organs.

The examination itself is very simple. The subject is asked to cover with one of the hands his ipsilateral nostril, to close his eyes, and while the examiner holds a test tube containing a material of characteristic odors (ground coffee, vanilla, tobacco, etc.) under the subject’s nose calling upon him to take several sniffs. The person is then asked whether he sensed a smell, and if so, to describe it. After the response the subject is asked to close the other nostril, and the same test tube is held under his nose. It is then asked if he can recognize the material on the basis of its smell. It is essential whether he judges the odors to be of the same strength on both sides. The subject's eyes are closed during the whole examination. Care should be taken that the odor samples must be characteristic materials that can be recognized by everyone, that they should be of adequate strength for recognition, while the smell should not be offensive. Generally, with the exception of certain specific situations, we should avoid using materials with strong odors and/or irritating nature.

After the examination, the examiner's **decision** is briefly summed up: 'All tested odors are sensed equally and named correctly in both nostrils, the patient does not report any pathological sensation of smell'.

1.4.1.2. The 2nd cranial nerve

The function of the second cranial nerve can be assessed from the results of several examinations. These are:

a. examination of the visual acuity

b. examination of the visual field

c. examination of the optic fundus

Before the examination we should ask whether he has any visual disturbance, whether he has glasses, and if so, what are the refractive powers of the lenses.

Examination of visual acuity or sight-testing

This examination is carried out by the help of the Snellen's test. For this test, a wall-table is used which contains a series of images (forks, letters, numbers, etc.) arranged in diminishing size downwards from above, which are seen at a visual angle of 5' from a given distance (50, 40, 30, 10… m), whereas the details of the images are seen at an angle of 1'. The subject sits or stands 5 m from the paper table in the practical hall or 20 feet from the illuminated table in the practical hall and covers one

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of his eyes with the ipsilateral hand. The subject is asked to read the sign that the examiner points to (or to indicate the direction of the opening of the fork or ring). Proceeding downwards from the top we want to find the smallest sign that is clearly recognized by the subject.

The visual acuity (visus) of the eye will be given by the number that we obtain as a quotient of the subject's distance from the table (5 m in the present case) and the distance from which a patient with appropriate eye-sight could read the smallest letter which was clearly recognized by our patient. In case of the illuminated table the value of the visus is written next to the smallest letter that is clearly recognized by the subject in a form of a ratio which could be used if the examination has been performed from 20 feet. If the distance of the subject from the illuminated table is not 20 feet then the actual distance should be used in feet in the numerator of the ratio instead of the 20. The visus is 1 in the case of good eyesight.

It may occur that the subject cannot see even the topmost figure, or contrarily, can read out the whole table ("supernormal" visus). After moving the table closer in the first case and farther away in the latter case, another trial can be performed to assess the patient's visual acuity. If reading the table fails to bring any result, the examiner tries applying the 'reading-the-fingers' test at various distances. When the subject is incapable of solving even this task, we must examine whether he can distinguish light and darkness. As the result of our examinations the vision of both eyes should be given separately. When the visus is not measurable, the visual performance should be described ('capable of reading of fingers at 2 m').

Examination of the visual field

The aim of the examination is the delimitation of the visual field, localization of possible scotomas ("blind regions" in the visual field), and determination of their size. The examination is performed in the following way:

A small-sized object is moved from outside the subject's visual field towards the center, and the subject notifies the examiner as soon as he perceives it. If the examination is repeated from different directions, the visual field can be mapped by connecting the points of perception. If the examiner moves the object up to the center of the visual field, possible scotomas can be mapped on the grounds of the subject's information. The examination must be performed, in accordance with the rules for paired sensory organs, separately for both eyes, hence the eye not examined at a given time must be covered by the patient with his ipsilateral hand. During the examination the subject fixes his position looking straightforward. The examination can be performed with the aim of orientation without any instrument (confrontal examination) or more accurately by means of a special instrument, the perimeter.

Confrontal examination of the visual field

The basic principle of this examination is that the examiner compares his own visual field with that of the subject, and gives an opinion on this basis. Stand face to face with the person to be examined and ask him to cover one of his eyes with his ipsilateral hand and fix his gaze on the forehead of the examiner. The examiner must

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also close one of his eyes, and fix his gaze on the subject's forehead, as well. The distance between them should be about 0.5-1 m. The subject's right eye is always examined by the examiner's left eye and vice versa, since when facing each other their contralateral visual fields correspond to each other. The examiner extends both arms sideways in a horizontal position (the index- and middle fingers straightened, the others bent), so that the hands are positioned in the **halving plane** of the imaginary line connecting the two open eyes. The subject is instructed to signal if he catches sight of the examiner's fingers on either side. Then the examiner begins to move one of his hands towards the center, in the halving plane, moving the index and middle fingers very quickly, as if he was going to drum with outstretched fingers. The basic rule is that the arm moves slowly, the fingers quickly. As the subject signals that he can see the examiner's fingers (in order to avoid dissimulation, it is advisable to ask about the direction too) the examiner judges how this point refers to his own visual field.

A difference of 1-2 cm cannot be delineated with this method, it is a difference of 15-20 cm of the visual fields that can be evaluated. The examination is performed, after the horizontal examination, on both sides in a vertical direction, then in the oblique directions too, i.e., each eye is examined from 8 directions. Then comes the examination of the other eye. The examination can only be evaluated if both sides keep the rules of implementation accurately, which must be continuously checked by the examiner.

Possible errors: either the examiner or the subject does not fix his gaze accurately during the whole course of the examination, opening of both eyes by either of the parties, the examiner's hands do not move in the bisecting plane, or the speed of motion is not correct, etc.

In the case of normal visual fields, the **diagnosis** is given as follows: 'The visual fields were found normal by confrontal examination'. When any impairment of the visual fields is found, it must be described in which quadrant of which eye the scotoma can be observed. In this case, examination with the perimeter is firmly indicated.

Examination of the visual field with a perimeter

The perimeter is an instrument designed to determine the visual field with high accuracy. In our practical exercises we shall use the arch perimeter. We can see on the instrument two scales graded in degrees. One scale is on the arch itself, the other one is on the axis of rotation. The former scale shows the extension of the visual field in the direction that can be read on the second scale.

The chin of the subject is placed onto a chin-support (in the most comfortable position for the subject), which can be fixed with screws so that the eye under examination will be at the level of the central point of the measuring arch. The other eye is closed, and the subject fixes his gaze on the white spot denoting the center of the arch. After checking whether all these conditions are met, the arch is turned in the desired direction. A white slip of paper is fixed into the slide of the instrument (or a slip of the necessary color if the color visual field is examined), then the paper is

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moved to the outermost position with the help of the screws on the slide. Then the paper is slowly moved towards the center until the subject signals to say that he can see the moving spot, i.e., the paper has entered his visual field. Then the values read on the scale are written onto the enclosed diagram of visual field examination. On this chart, one can see concentric circles symbolizing the 180 degrees of the visual field with directional graduations of 360 degrees. Also shown on the chart is the physiological boundary of the visual field drawn in a line. Two separate diagrams are used for the two eyes. The examination is performed by visual-field directions of 10-15 degrees first on one, and then on the other eye. The borders of the visual fields under study are plotted by connecting the recorded points.

Examination of the optic fundus

Examination of the optic fundus is performed with an ophthalmoscope. The examination is based on the principle that the light entering the eye through the pupil is not fully absorbed but is, in part, reflected, and leaves the eye.

If somebody's eye is illuminated with an appropriately intense light, the reflected light may be enough to make the fundus of the eye visible. Since the rays of light coming out of the eye are refracted when passing through the lens and the cornea, their focusing into a clear-cut image is possible only in certain special cases; namely when the joint refracting power of the two eyes (those of the examiner and the subject) makes it possible. This condition can be most simply satisfied when both persons involved in the examination focus on infinity (in other words, they both try to look far away). In this case, the rays of light leaving a certain point of the fundus are made parallel by the lens on passing, and these parallel rays will be united again by the eyes of the examiner **focused on infinity**. Focusing the subject's eyes on infinity can be most practicably implemented if we ask him to stare, during the whole examination, at a point-like object at the longest distance allowed by the circumstances, at medium height. This object may be a door handle, electric switch, the corner of the room, or even a window of a neighboring house. The only important thing is that it should be as far away as possible, as small as possible, but still well visible.

The basic rule is that the examination should always be performed with the **ipsilateral eye**, (i.e. left with left, right with right), and in the meantime, care is taken that the examiner's head does not obstruct the sight of the target of the eye which is not examined currently. Focusing our own eyes for infinity during the examination is a central task to be learned. The most feasible way is if we try not to look into the inner side of the subject's eye, we must act as if it was a hole through which we want to look far behind him (similarly to the situation when using a microscope). If done properly, the examiner can see a light orange spot, on which, in fortunate cases, several bright red branching lines might be seen, which are the blood vessels of the retina. If one of these blood vessels is followed, (always proceeding towards the centre of the fundus), sooner or later the **optic papilla** will become visible. At this point the entry and exit points of the blood vessels and lateral to the papilla, the yellow spot (**macula lutea**) may also be seen. It must be noted, however, that it takes

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considerable practice to observe all the introduced elements of the fundus, and at the beginning of the investigation it is good enough to see the reddish image of the fundus only.

Nevertheless, in a healthy eye, the papilla has sharp edges, and its physiological excavation can be clearly observed. The blood stream in the vessels is steady, there are no deposits, stenoses or other pathological disorders in their walls.

In order to alleviate the examination, the ophthalmoscope contains several accessories. The color and intensity of the illumination as well as the size of the illuminated area can be altered. The combinations of these factors can be set by the regulator rings on the handle. Naturally, if either the examiner or the subject has any refractive error (in a simplified way: they do or they are supposed to wear glasses), the above way of examination will fail to give a well-defined fundoscopic picture. In order to eliminate the error introduced by the refractive error(s), a series of corrective lenses are built into the device, which serve to compensate for the refraction error(s) (within the range of -50 to +50 diopters). At the beginning of the examination, the examiner should ask whether the subject wears glasses, and if he does, what are their refractive powers. Do not forget that not the glasses but the eyes should be corrected. The refractive error (ametropia) of the eyes corrected by eyeglasses with negative sign is positive. If the eyes of both the examiner and the subject are ametropic, it is the **sum** that should be considered for correction. When the value of ametropia is identical but has opposite signs, no correction is needed. (It may be mentioned, although it is irrelevant from the point of a neurologist, that ophthalmoscopy is suitable for the objective determination of the refractive errors as well, so that the examiner sets, by the help of the series of lenses, a well-defined fundoscopic picture, then reads the degree of the correction applied.)

The **results** of the examination of the 2nd cranial nerve is summarized in the following way: 'Intact visual acuity bilaterally. The confrontation test does not reveal any defect in the visual fields. On the optic fundi the disc edges are sharply defined. The color of the disc is pink and the size and regularity of the retinal vessels are normal. No abnormal retinal pigmentation.'

1.4.1.3. The 3rd, 4th and 6th cranial nerves

The function of these cranial nerves and that of the ocular muscles innervated by them can only be examined jointly. Under physiological conditions, the state of the separate anatomical units is not detailed, our diagnosis of the ocular movements is given in a complex form. In the case of suspicion of a pathological disorder, the suspected area is delineated by task-oriented examination, and in the diagnosis it is noted in an emphasized form. The following examinations will be performed:

a. function of the internal ocular muscles on the basis of judging

- the shape of the pupil
- accommodation
- convergence response
- direct response to light
- consensual response to light

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b. function of the external ocular muscles by examining

- lifting the upper eyelids
- ordered, guided and searching eye movements.

Examination of the internal ocular muscles

The examination begins with the observation of the pupils. The pupils of a healthy person are round, with sharp margins, they are located in the center of the iris, their diameters are equal, and they show no spontaneous movement.

In the course of **accommodation,** the pupils should constrict. To perform the accommodation test, put your right hand on the subject's head to fix it. Hold your left hand, with straightened index finger, in front of the subject reasonably far away (a distance of 50-60 cm is perfectly appropriate). Ask the subject to look at the tip of your index finger, and in the meantime observe the pupils. Move your left hand towards the subject's eyes fairly rapidly (NOTE: when the accommodation reaction is investigated the finger should never be brought closer than the near point [15-20 cm from the eyes]). Due to (physiological) accommodation the pupils will slightly constrict.

To elicit the **convergence response**, fix the head of the subject with the right hand. Ask the subject to fix his gaze on a far object (situated farther than the near point) in front of him. In the next step move your extended right index finger from the direction of the forehead into the patient's visual field. Note that your finger should be about 5-6 cm from the eyes (so it should be within the near point), and order him to look at your index finger as soon as it enters his visual field. You may repeat the investigation several times by withdrawing your finger from his visual field, and asking him to look at the previously defined far object again. When looking at your finger, the axes of the eyes should converge (this requires the proper function of the extraocular muscles) and the pupils constrict.

When eliciting a **direct response to light**, cover one of the eyes and illuminate the other one with a low intensity light. The pupil of the illuminated side should constrict. When the light is not applied, the pupil will (slowly) resume its original size. When eliciting the **consensual response**, the non-illuminated pupil is observed, which should also constrict as a result of the light stimulus applied to the other side.

Examination of the external ocular muscles

When observing the function of the external ocular muscles (as an exception, both members of the paired organ are examined together), first, we observe the upper eyelids. Since the levator palpebrae muscles are innervated in part by the third cranial nerve, drooping of the upper eyelids (ptosis) might be a sign of dysfunction of that nerve.

Following this, the subject is asked to look around and find specific objects in his environment without moving his head (**searching eye movements**). In this case the patient is usually asked to find a specific object in the environment, whose position he can find by moving his eyes only (i.e. look at the fridge, look at the tap,

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etc.). When the searching eye movements are investigated, no specific order should be given (such as 'look to the left to find the fridge', as this is going to induce ordered, rather than searching eye movements; see in the subsequent step). In the next step, the patient is ordered to look up, look down, look to the left and look to the right for a longer period of time (**ordered eye movements**). Finally, the examiner moves his right index finger in front of the subjects face at a distance of about 30 cm, and instructs him to follow the movement of the finger with his glance without moving the head (**guided eye movement**). By moving our fingers the eyes must be directed to their most extreme (most marginal) positions, in such cases the examiner waits for a few seconds and asks the subject whether he has any double vision or vertigo. Note that (if present) nystagmus most likely occurrs when the eyes are in their most most marginal positions; or if nystgamus is present even without guiding the eyes to the marginal positions, it is expected to become more severe near or at the marginal positions. In the meantime, the examiner's hand should be placed on the subject's head, assuring its fix position. A short delay must be inserted between the individual commands, and the right hand must be moved slowly, so that the subject has time to respond. During the whole length of the examination, the eyes must be observed, so that we can judge whether the subject is capable of:

- fulfilling the orders completely,
- looking at any direction,
- fixing his glance in the given direction even in an extreme position.

The eyes must be observed carefully, the examiner is to search for pathological ocular movements, such as nystagmus. If there are such phenomena, it must be described when they appear, how long they persist, and what their directions are.

Finally, the examiner draws the **conclusions** (if each performance examined was found to be physiological): 'The pupils are round, equal in size, and 3-4 mm in diameter and have sharp edges. The pupils react to light equally, both directly and consensually. The pupils react to accommodation, the convergence of the axes of the eyes is bilateral and equal. There is no eyelid retraction or ptosis. Extraocular movements are intact in all directions of gaze. Nystagmus cannot be observed. Double vision (diplopia) is not reported.'

1.4.1.4. The 5th cranial nerve

The 5th cranial nerve is the general sensory nerve of the face and the motor nerve of the masticatory muscles. Examination of this nerve includes the examination of the exit points, and sensations of heat, touch, pressure and pain. In addition, the performance of the masticatory muscles and the cornea reflex must also be evaluated.

On examining the **sensory function**, the subject has to be able to recognize a given stimulus (cold, heat, prick etc.), then signal whether he can sense the stimuli of equal intensity as equal in strength in the different regions of the face. Each division must be compared to all the others, so that we can reveal not only the total loss of function, but an impaired function as well. Thus, e.g., if a thermal stimulus is applied to the left side of the forehead, and if the subject can recognize it, the examiner

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proceeds to the same region on the right side. If the subject recognizes the stimulus type, the examiner immediately asks whether he has felt it equal to the previous stimulus. Note that the sensations must be compared between those regions as well, which are innervated by the different branches of the trigeminal nerve belonging to the same side. The following functions are examined:

a. sensitivity of the exit points
b. cornea reflex
c. motor function
d. sensory function
e. m. masseter reflex

Examination of the exit points

This examination is simply performed by exerting pressure on the cranial exit points of the three main divisions of the nerve with our thumbs. The subject should not indicate spontaneous pain. In physiological cases, sensitivity to pressure (meaning unduly strong pain) is not encountered either.

Cornea reflex

The essence of the reflex is that mechanical stimulation of the cornea evokes the closure of the eyelids. It can be most simply elicited by a long, pointed roll of cotton. Approach the cornea always in a way that the approaching object is not seen by the patient (i.e. he is ordered to look to the left and the object is approaching from the right), otherwise, the oculopalpebral reflex (reflexive closure of the eyelids) will be evoked by the object appearing suddenly in the visual field, and this may lead later to erroneous diagnosis. Remember at the evaluation of this test that the prerequisite of regular performance of this reflex is not merely the intact sensory function of the trigeminus, but also an intact motor activity of the facial nerve (eyelid closure, see later).

Motor function

When judging the motor movement, the first step is the examination of the mass and tone of the masticatory musculature, and the judgment of the possible deviation of the lower jaw. In pathological cases, this step itself can give a good deal of important information.

First, the subject is asked to open his mouth wide, and the position of the lower jaw is observed. Then he is asked to strain his jaw rightward and leftward, close his mouth tight, then open it and hold it in these positions. The head is fixed by the examiner's left hand, whereas the right hand tries to overcome the force of the muscle holding the jaw (open the closed mouth or bringing to middle position the jaw strained sideways). The strength of the healthy masticatory musculature cannot be (easily) overcome in this way.

Sensory function

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In this examination, the specific types of sensation must be checked one by one. Sensation of cold is examined by filling a test tube with tap water and touching the points to be examined. Sensation of heat is examined in the same way, but the test tube is filled with hot water. After filling, wipe the test tube dry for both examinations and press the tube against the point for a few seconds. Avoid using extreme temperatures. In the sensation of pain, the area to be examined is gently pricked with a pointed object. The prick should be a superficial pressure, never penetrating the skin. Tactile sense can be examined with a piece of fine cotton wool with a pointed end. The area to be examined is gently touched with the cotton, and the examiner asks whether the subject has sensed anything. The pressure sense can be most simply examined with a blunt tool (e.g. a penholder). In addition to the examination of simple pressure sense, one has to find time to test dermolexia. This consists of writing letters or figures with a blunt tool on the subject's forehead and face. Make sure that the letters are written to those parts of the face, which are innervated by the different branches of the trigeminal nerve (and therefore six letters should be written altogether). In the case of normal sensory function, the subject should recognize the letters (or numbers). Only one letter or figure should be written at the same time, and it must be larger than 4 cm.

M. masseter reflex

This stretch reflex can be evoked by a hit using a reflex hammer to the thumb of the examining person placed on the jaw of the subject. This should be done in a slightly open position of the mouth and the response is the rapid closure of the mouth due to the rapid contraction of the masseter muscles.

If there is no pathological sign, the findings can be **summarized** as follows: 'Exit points of the trigeminus are not sensitive to pressure, cornea reflex is moderately vivid on both sides, sensory function (heat, pressure, touch, pain) is normal in the area of all three branches; the subject recognizes the numbers written on its face; mass, touch, strength of the masticatory musculature are normal, no deviation of the lower jaw on opening the mouth; the m. masseter reflex is moderately vivid.'

1.4.1.5. The 7th cranial nerve

The 7th cranial nerve is the general motor nerve of the mimetic musculature of the face, and the gustatory nerve of the frontal two third of the tongue. These functions will be examined separately as follows:

a. motor function

b. sensory function

Motor function

The first step in the examination is observation of the face. Observe the run of the labial and nasolabial sulci, the eyelids, and the palpebral aperture at rest. Asymmetry of face, oblique line of the mouth are indicators of motor function

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disorders. After this inspection, the subject is instructed to close his eyelids, to frown, show his teeth, whistle, and the examiner observes whether the implementation is complete. In the meantime, the strength of the musculature is checked by palpation, and the appearance of asymmetry of the face or any pathological form of motion is noticed during the performance.

Sensory function

The examination of **gustatory sense** is performed separately for the five taste qualities (umami (during the lab tasteless is examined instead), salty, sweet, sour, and [always at the end] bitter). It is especially important in this examination that the course of the test should be previously described for the subject in detail, otherwise he cannot effectively participate in it. The subject is given a chart with the names of the primary tastes listed, and he is ordered to put out his tongue. The taste sample is applied onto the apical two thirds of the tongue either on the right or the left side, with the help of a piece of cotton rolled on a wooden stick. The subject, without withdrawing his tongue, points to the name of the recognized taste on the table. If withdrawn, the tongue may be contaminated in the oral cavity by other tastes or the taste sample applied may get outside the desired area, rendering the examination pointless.

After all that, the mouth is rinsed with clear water, the subject puts out his tongue again and the examination is repeated with the same taste on the other side of the tongue. If the subject can recognize the taste on both sides, the examiner asks him whether he sensed it to be of equal strength on both sides. **Note that it is a fundamental rule that one stick must be used only once.**

If the movement of the mimetic musculature is sound, the basic primary tastes were recognized and found to be of equal strength, the **opinion** can be summed up as follows: 'No asymmetry of face at rest. Frowning, closing of eyelids and showing the teeth were performed with equally good strength on both sides. The subject can recognize the tastes on the frontal two thirds of the tongue and signals them to be of equal strength.'

1.4.1.6. The 8th cranial nerve

Since the 8th cranial nerve has a double function, through the examination of this cranial nerve, the auditory sensation and the sense of balance are examined. The examination of the vestibular organ is closely related to that of coordination, for that reason it is dealt with in Chapter 1.4.5. To judge the **auditory sensation,** the following examinations have to be carried out:

a. Hearing test with whispered speech

b. Tuning fork tests

- Rinne's test
- Weber's test
- Schwabach's test

c. Audiometry

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Hearing test with whispered speech

The examination can be performed very easily, without instrumentation. Stand at a distance of 6 m from the subject, who should stand sideways. He is asked to cover his contralateral ear with one of his hands. The examiner whispers short words which have to be repeated without mistake by the subject. Only accurate repetition can be accepted. Signaling simply by saying 'I can hear' or 'I cannot hear' should not be accepted. It is important that the whispered words must be uninflected and unambiguous. Equivocal or rhymic words should be avoided (big-dig, fall-wall).

If the subject does not recognize whispered speech from 6 meters, the test can be repeated from a shorter distance. Finally, the diagnosis refers to the distance at which recognition is exact: 'the subject recognizes whispered speech at 4 meters'.

Tuning fork tests

The purpose of the **Rinne's test** is to compare the subject's air conduction of sound to his bone conduction. In a healthy subject, the air conduction is better then the bone conduction. To perform the test, hold the vibrating fork with its shaft firmly on the mastoid process, ipsilateral to the tested ear, and ask whether or not he can hear it. It is insufficient to place the fork gently, because the quenching of skin does not allow conduction with high efficiency. If the subject can hear the vibration, wait until sensation of the sound disappears (because of quenching). When it is no longer heard, transfer the fork to a position near the outer ear, and in this case the sound should be heard again ('Rinne positive'). If the subject does not signal that he can perceive sound vibration, air conduction is weaker than bone conduction ('Rinne negative'). It is advisable to repeat the test in the opposite order as well; in this case the tuning fork should be held near the outer ear, and when the subject indicates that he cannot hear the vibration, the tuning fork should be immediately transferred to the mastoid process. Under physiological conditions, the patient should not hear anything after transferring the tuning fork to the mastoid process. Naturally, Rinne's test should be performed on both sides.

The aim of the **Weber's test** is to compare bone conduction of the two ears. Rest the base of a vibrating tuning fork on the vertex of the subject's skull. The subject is asked where he can perceive the sound vibrations (which will reach the organ of Corti by bone conduction). If bone conduction is equal on both sides, the diagnosis will be 'Weber infinite'. If the subject perceives the sound louder on one side ('Weber's test deviates to this side') that indicates problems with either the air conduction (conductive deafness) or lesion of the nervous apparatus attached to the auditory system (sensoryneural deafness). Interestingly, in an air-conductive disturbance, Weber's test will deviate to defective side. In such a case, Weber's test is informative only after comparison with the result of Rinne's test. If the ear where Weber deviates to is Rinne positive, then a sensoryneural disturbance of the contralateral ear is likely. If the ear where Weber deviates to is Rinne negative, the air-conductive disturbance of the same ear is likely. This latter phenomenon can be emulated by blocking one's external auditory canal, thereupon, the sound will be louder.

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The goal of the **Schwabach's test** is to compare the auditory threshold of the subject to that of the examiner (which is supposed to be normal). Strike a tuning fork and hold it a few centimeters away from the subject's ear. The subject is asked to signal, when the vibration cannot be heard any more. Then the fork is moved to our own ear by checking whether we can or cannot hear the sound. There is a chance for that, based on this examination, the examiner's ear will be the weaker. In this case, repeat the test with vibrating and non-vibrating fork randomly, to exclude occasional dissimulation. The Schwabach's test should be performed for the bone conduction as well, and the examination obviously should be done on both sides. There is no reason of performing Schwabach's test with diagnostic aim if objective audiometry is possible.

After the examination, the examiner's **decision** is briefly summed up in normal case: 'The subject is capable of hearing whispered speech at 6 meters, Rinne's test is positive on both sides, Weber's test is infinite and Schwabach in normal on both sides'.

Audiometry

The purpose of audiometry is the objective measurement of the auditory function. Audiometry is usually performed in an audiometry chamber, in order to keep conditions strict. During the examination, auditory thresholds of both ears are determined on the whole scale of hearing at 10-12 different frequencies. Earphones are placed on the subject's ears, and the intensity of sound is gradually increased (on different frequencies), while the subject signals when he can hear the sound. The threshold intensities, expressed in decibels, are plotted as the function of frequency on a graphical scale. The measurement must be arranged so as the subject cannot see the operating board of the setup. It is very important that the subject examined must signal with his hands, but never vocalize, because of the interference with his own voice. Current audiometers are constructed with remote control for the patient to signal if the sound is perceived. As a matter of fact, there is no need to indicate in which ear the tone is sensed. Since laterality could be set on the operating board of the audiometer, only yes or no response is necessary.

1.4.1.7. The 9th cranial nerve

The 9th cranial nerve has mixed functions, and the following features are examined:

a. Taste sensation on the posterior third of the tongue
b. Sensory function of the upper part of the pharynx
c. Motor function of the upper part of the pharynx

Examination of taste sensation

The examination of the posterior third of the tongue is similar to the process described for the 7th cranial nerve.

Examination of sensory function

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Sensory function can be examined by eliciting the reflexes of the pharynx and the palate. The subject is asked to open his mouth. Touch the soft palate, the pharyngeal arch and the posterior wall of the pharynx on both sides using a spatula. In normal cases, the pharyngeal muscles contract, or even a movement of defense can develop (e.g. pulling back of the head). Do not apply too strong stimuli when the reflexes are elicited. It is usually enough to touch the surface of the pharynx.

Examination of motor function

Before starting the examination of motor function we have to inquire thoroughly whether the subject has any difficulty with swallowing, phonation or speech. Thereafter, inspect the open oral cavity and observe the position of the soft palate, the pharyngeal arches and the uvula. Asymmetrical pharyngeal arches, hanging soft palate or slanting uvula suggest pathological motor function. If the subject does not report any difficulty with swallowing or phonation, he is asked to eat a few bites of food, and then swallow 1-2 sips of drink in the presence of the examiner. It must be noted that long before starting the examination of the 9th cranial nerve, the experienced examiner notices even the relatively hidden difficulties of phonation during communication with the patient. Rough insufficiency of sound formation is obvious even for the ordinary observer. If the subject complains of frequent dysphagia, do not perform the swallow tests. If the subject does not report any sign of dysphagia, first examine his capability of swallowing compact material. If any dysfunction can be noted, disregard fluid swallowing.

If each performance examined was physiological, the examiner draws the **conclusions**: 'Soft palates are proportionate. The uvula does not deviate. The reflexes of the soft palate and pharynx are of medium intensity on either side. Swallowing and sound phonation are undisturbed. The subject can recognize the tastes on the posterior third of the tongue and signals them to be of equal strength.'

1.4.1.8. The 10th cranial nerve

The 10th cranial nerve plays role in the motor innervation of the larynx, pharynx and esophagus. The examination of these functions is carried out together with the examination of the 9th cranial nerve. Vegetative function of the 10th cranial nerve (stomach emptying, negative tropic effects) are beyond the general nervous system examinations.

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1.4.1.9. The 11th cranial nerve

The function of the internal branch of the 11th cranial nerve is examined together with the 9th and 10th cranial nerves in the swallowing and speech tests.

The external branch of the 11th nerve provides motor innervation of the sternocleidomastoid and, partly, the trapezius muscles. During the examination of this division, the performance of the above muscles is tested. First, touch the muscles, judge their mass and tone. After this, ask the subject to elevate his shoulders, to turn away and bend his head, and then, to hold his head steady in these positions. The examiner tries to bring the subject's head back to the original, medium position. Under physiological conditions, the examiner, applying medium muscle strength, fails to overcome the strength of the subject's examined muscles.

The **opinion** can be summed up as follows: 'Mass and tone of the sternocleidomastoid and trapezius muscles are normal. Shrugging of shoulders, turning away-, bending- and inclination of the head are performed with suitable force.'

1.4.1.10. The 12th cranial nerve

The 12th cranial nerve is the motor nerve of the tongue. During the examination of the nerve, the movements of the tongue are studied. First, the subject is asked to put out his tongue and the examiner inspects it. In normal cases, the tongue is symmetrical and the peak does not deviate, involuntary twitches, bite marks and atrophy cannot be observed. Then the subject is asked to move his tongue in the horizontal and vertical planes (bend the tongue to right, left etc.). The examiner judges whether the movements are not obstructed.

The **decision** is summarized: 'The protruded tongue does not deviate, its movements are unobstructed, atrophy, fasciculation (involuntary movements) and bite marks cannot be observed.'

1.4.2. Examination of general motor function

In the examination of the locomotor organs, we wish to gain information on the organ system of locomotion and its innervation. The two main parts of the examination are:

a. examination of trophism and tonicity

b. examination of muscular strength.

1.4.2.1. Examination of trophism and tonicity

This examination can be carried out most easily if the subject is in a supine position with his musculature relaxed. The muscles and groups of muscles are palpated and their state of development assessed. By moving the joints from one extreme position to the other, the tone of the muscles can be judged.

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1.4.2.2. Muscular strength

When examining muscular strength, first we examine the squeezing power of the hands, then the strength of the upper extremities and the lower ones.

In examining squeezing power, we stand face to face with the subject, stretch out our second and third fingers fitted to each other with crossed forearms and order him to squeeze them. In addition to assessing squeezing power, we must observe whether the subject squeezes on both sides with equal strength. Then follows the examination of ring-formation and finger stretching. The subject is asked to fit his thumb to his 2nd, 3rd, 4th and 5th finger, one by one, forming rings. The examiner makes then an attempt to open these rings. During the examination of finger stretching, the examiner attempts to close the stretched fingers of the subject. Then follows the systematic examination of the skeletal musculature. The basic principle is simple. The strength of all muscle groups moving the joints of the given extremity are tested successively at each joint, proceeding from the distal end to the proximal one. The subject is asked to fix the joint in an extreme position, the examiner tries to move it back to central position ('bend your elbow and do not allow me to straighten it', or 'stretch your elbow and do not allow me to bend it'). The examiner's left hand supports the joint, his right hand seeks a stable grip in distal direction and tries to overcome the strength of the muscles. On the lower extremity the examination begins with the big toe, on the upper extremity with the wrists.

If the examiner finds normal conditions, his **opinion** is as follows: 'Mass, palpation and tone of the skeletal muscles are normal, muscular strength is sufficient for all the body. Atrophy and fasciculation (involuntary movements) cannot be observed.'

1.4.3. The sensory system

Examination of the sensory system consists of the following tests:

a. superficial perception and dermolexia
b. depth perception
c. stereognosis

1.4.3.1. Superficial perception and dermolexia

The determination of superficial perception and dermolexia is performed in the same way as described for the trigeminus, keeping in mind that each dermatome has to be examined separately.

1.4.3.2. Depth perception

Information on depth perception can be obtained by testing body sense and sensation of vibration. To test body sense, the subject is asked to close his eyes and bring a particular joint to the middle position. The examiner gently turns aside the limb and the subject must tell the examiner which of the joints was moved and what was the direction of the displacement ('bending the index finger' or 'forcing the wrist back'). The sense of vibration is examined with a tuning fork pressed against the

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joint. The subject must name the joint to which the vibrating tuning fork is pressed, and signal whether he senses the vibration.

1.4.3.3. Stereognosis

Stereognosis is the recognition of an object by touch or feeling its shape. During the examination, the subject closes his eyes, a small object (key, pen, eraser, or lighter) is given into his hand, and he must recognize the object by touching.

When sensory function is assessed in the whole body of the subject, the examiner summarizes his **findings** as follows: 'The subject senses the prick of the needle, touch with cotton wool, cold and heat all over his body, and signals them to be equal, localizing power and movement in the small joints, sense of vibration and dermolexia are maintained throughout the body, *subjective sensations (spontaneous pain, paraesthesia) are not present*.'

1.4.4. Reflex action

Examination of the various reflexes, yielding a great deal of information, is carried out within the reflex system. In the frame of the present examination, the following reflexes must be investigated:

a. stretch reflexes

b. superficial reflexes

1.4.4.1. Stretch reflexes

Stretch reflexes (or tendon reflexes) are evoked by using a reflex hammer. The subject is in supine position, his limb is brought into a middle position and a slight blow is delivered on the tendon of the muscle tested, or on the bone to which the muscle is attached. Not only the strength of the elicited reflex is observed, but the reflex time as well.

In the case of the **biceps jerk**, the blow is not applied directly on the tendon, but the examiner places his index finger or thumb onto the transition between the muscle and tendon, and the tendon is gently pressed towards the humerus. The examiner strikes a blow with the hammer on his own finger then, which elicits the reflex action. In the case of the **triceps jerk**, the blow is struck above the semiflected elbow joint on the tendon of the triceps muscle, and the result is the extension of the elbow joint. The **radial jerk** can be activated by hitting the processus styloideus radii. The reaction will be a mild flexion. The **ulnar jerk** requires a slight pronation of the hand, and the blow is struck on the ulnar capitulum. The result will be a mild pronation, occasionally with a little extension. When eliciting the **patellar jerk**, the extension of the semiflected knee can be observed, if we strike the patellar ligament. If the reflex is torpid, the examiner places his hand on the quadriceps femoris muscle, then even the smallest movements or twitches can be observed. To evoke the **Achilles jerk**, the knee joint is bent at right angle. The examiner slightly dorsiflects the foot with his left palm from the direction of the sole and strikes the Achilles

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tendon. The evoked plantar flexion, even if it is invisible, can be surely sensed as a fine pushing movement exerted on the examiner's palm.

1.4.4.2. Superficial reflexes

To elicit these reflexes, blunt needles are used. When eliciting the **abdominal skin reflex**, the needle is drawn along the skin starting from the edge of the abdomen towards the navel. Make sure that the stimulus is one, firm scratch, rather than fine stimulation. The stimulus (if applied appropriately) will induce the navel to move towards the needle. The examination is performed from two horizontals and four diagonal directions. When eliciting the **plantar reflex**, the blunt needle is drawn along the medial edge of the sole, which causes plantar flexion of the toes.

1.4.5. Examination of coordination

The term ‘coordination’ refers to coordination of the motor movements. Before starting the examination, the subject has to be observed in a resting position. We must observe whether he shows any pathological motion forms, whether we can observe trembling, and problems with the posture or nystagmus.

Disorders of coordination may be evoked experimentally, if the subject is turned around his axis for a half or one minute, then the examination is performed shortly after the subject has stopped turning. In such cases, so-called post-rotatory nystagmus may be observed, or past pointing in the Bárány's test (see later). The following examinations are performed:

a. target tests
b. Bárány's tests
c. diadochokinesis
d. rebound phenomena
e. Romberg positions
f. walking with closed eyes

1.4.5.1. Target tests

In the target test, the subject in supine position is instructed to close his eyes and put his right index finger on the tip of his nose (**finger-nose test**) then place his right heel on his left knee and slide it slowly down along the tibia (**knee-heel test**). Then the examination is repeated with the left heel and right knee. A subject with proper coordination can perform the tasks easily with closed eyes. In the case of impaired coordination, the subject cannot perform the task at all, or only after several failed attempts. Note that the two tests are separately performed, and that the knee-heel test must be performed while supine, never while standing.

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1.4.5.2. Bárány's test

The Bárány's test can be best performed in standing position. The examiner stands face to face with the subject at such a distance that, with the arms stretched out straightforward, the fingers can come into exact overlap. The examiner's fingers are above, the index and middle fingers are stretched out, while the others are closed. The subject's task will be to close his eyes and lower his arms alongside his body, then lift them up again below the examiner's fingers, hitting them upwards a little. The examiner observes whether the subject is able to bring his fingers back to the original position (just below the fingers of the examiner) or blunders in finding them. A person with sound coordination can perform the task without failure.

1.4.5.3. Diadochokinesis

When examining diadochokinesis, the subject must always be shown the task, so that he can understand what he is expected to do. The task is twiddling with the fingers, and alternating pronation and supination of the wrists at the highest possible speed. Let us observe what is the highest speed the subject can reach, and how often he makes mistakes. Disturbance in implementation or complete loss of coordination is called **dysdiadochokinesis** or **adiadochokinesis,** respectively.

1.4.5.4. Rebound phenomenon

The examination of the so-called rebound phenomenon usually takes place after the previous tests. The subject is ordered to flex his arm. With the open palm of his left hand the examiner supports the subject's ipsilateral shoulder, while the right hand of the examiner grasps the subject's wrist (or closed fist) exerting a strong pull to the forearm, as if he wanted to straighten the elbow. After three or four seconds of pulling, the examiner ceases the pulling abruptly. The forearm of a person with normal coordination does not slam back above the shoulder more than 1-2 cm. In the case of a severe coordination disorder, rebound phenomenon may be so strong that the patient may injure his own shoulder (or face) when hitting it. This should be prevented by the examiner’s open left palm.

1.4.5.5. Romberg positions

The **classical Romberg position** is when the patient stands with his feet closed together completely, hands stretched forward with palms up and eyes closed. In the **aggravated Romberg position**, one foot is placed in front of the other, so that the longitudinal axis of the feet should be aligned. Standing in this position is impossible with impaired coordination, and the subject will lean in one or another direction. In anticipation of such a possibility, the examiner always stands close to the subject in order to prevent his falling down.

1.4.5.6. Walking, walking with closed eyes

In the course of the walking tests, it is observed whether the subject is able to walk straight, keeping their direction with open or closed eyes. The subject stands in front of us with his back towards us. He is ordered to walk forward (approx. ten steps)

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with open eyes then stop. We should observe whether he can keep a straight direction, and whether he has taken the given number of steps. To observe straight direction we can use the straight line formed by the tiles of the floor, or an object that was set as a target. It is observed whether the subject has gone astray from the straight direction, which way and to what extent. After that, he is ordered to turn back, close his eyes and walk back towards us with his eyes closed.

The **results** of coordination tests may be formulated in the physiological status as follows: 'The subject remains stable in Romberg position, walking with closed eyes the direction is kept, past-pointing is not present in Bárány's test, target tests are performed precisely, dysdiadochokinesis or rebound phenomenon could not be observed'.

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2. EXPERIMENTS ON ISOLATED ANIMAL TISSUES

Experiments performed on isolated animal tissues allow the investigation of physiological phenomena under almost physiological conditions. Thus, drug effects or effects of changes in certain parameters may be studied under controlled circumstances. Training in the **proper use of the electrical and surgical instruments**, applied during these experiments, gives a general insight to laboratory work. In addition, it also helps to develop **manual skills** necessary for the everyday medical practice.

2.1. Properties of the blood

The most important points of the physiology of the blood are described in textbooks of physiology for medical students, and they can also be found in different textbooks; the authors strongly suggest a brief overview of the relevant chapters before commencing the experiments described below.

2.1.1. Handling of blood samples

The experiments on blood samples are conducted on fresh blood yielded from animals. Although the animals are checked by a veterinarian, and thus the blood does not contain infectious agents, application of certain elementary safety measures (wearing white coat, using medical gloves, washing hands frequently, carefully cleaning the tools and keeping general order) are strongly recommended.

2.1.2. Cleaning the tools

Some of the tools are used repeatedly, therefore, they are rinsed with tap water, followed by distilled water, and then dried with a clean tissue. Mélangeur pipettes and osmometers are washed in the same sequence (tap water, distilled water) using vacuum (water-pump). Water-free acetone and air is flushed through for rinsing and drying. The pipettes are ready for use when the glass bead rolls freely with no tendency to adhere to the side of the bulb. KOH solution is also available; however, since it precipitates blood, it can only be used following a rinse with water. **Organic solvents (benzine, xylol, toluol) or alkali containing detergents must not be used for cleaning plastic tools, only distilled water should be used to clean these.**

2.1.3. Transport properties of red blood cell membrane

The solutes of the solutions used during the lab may traverse the cell membrane with the help of different mechanisms, down their concentration gradients. Among the investigated substances, thiocarbamide and glycerol are lipid soluble, therefore, they can easily penetrate the membrane. Glucose entry is mediated by facilitated diffusion which has a relatively low transport velocity and can be described with a transport maximum. NH_4^+ cannot directly enter the red blood cells; however, in

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solution it is in dynamic equilibrium with NH_3, which – being gas and lipid soluble – can freely permeate cell membranes (non-ionic diffusion) and will then be reconverted to NH_4^+ in the intracellular space, and therefore unable to diffuse back again. The above mentioned osmotically active particles, using different mechanisms to get through the membrane, cause the water uptake of the cells by increasing the intracellular osmolarity which serve as a driving force for water entry. This process, in turn, leads to cellular swelling, and after exceeding a certain level, to the rupture of the red blood cell membrane, causing hemolysis.

The higher the transport velocity of the individual substances through the membrane, the sooner hemolysis occurs. Therefore, the rate of development of hemolysis is a good indicator of the velocity of the transmembrane transport. During the lab, we use red blood cell suspensions, and detect the intensity of the transmitted light at the wavelength of 540 nm using photometer. The light beam passing through the suspension will be scattered into several directions by the intact red blood cells, and therefore a certain portion of the entering light does not reach the detector situated opposite to the source of light. It is easy the understand that the higher the concentration of the red blood cells in the suspension, the smaller portion of the light can reach the detector, and therefore the extinction value becomes higher. As a consequence, if the concentration of the intact red blood cells decreases, the number of cells capable of scattering the light decreases, which, in turn, leads to a reduced extinction level.

Experimental procedure

Time-dependent changes in extinction are monitored using a photometer coupled to a recorder. The recording velocity should be 50 cm/h except for the experiment with thiocarbamide, where the value of 5 cm/min is more reasonable.

Step 1.: Before dilution shake the original blood sample well then dilute the blood sample 5-fold using physiological saline (0.2 mL blood plus 0.8 mL physiological saline)! Use this diluted blood throughout the experiments!

Step 2.: Add 100 µl of the 5-fold diluted blood sample to 3 mL of distilled water (blank) and another 100 µl to 3 mL of physiological saline (starting value)! The cells fully hemolyse in the sample suspended in distilled water, which leads to the decrease of turbidity. Adjust the zero extinction of the photometer to this value (as well as the baseline of the recorder). After this, determine the extinction of the blood sample suspended in physiological saline (which gives us the maximal extinction), and then set the recorder the way that the extinction of this sample does not exceed the measurement range of the instrument.

Step 3.: Pipette 3 mL of thiocarbamide (300 mmol/L), glycerol (300 mmol/L), glucose (300 mmol/L) and NH_4Cl (150 mmol/L) containing solutions into different test-tubes. Add 100 µl of the diluted blood sample first to the thiocarbamide solution, rapidly mix it, and quickly begin monitoring the changes of the extinction! The measurement will be performed until complete hemolysis is achieved, or at least for 6 min. Repeat the experiment using glycerol, glucose and NH_4Cl solutions as well! Note that the blood sample must be shaken vigorously before immersing the pipette!

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2.1.4. Determination of the osmotic resistance of the red blood cells

In certain types of anemia (e.g. sickle cell anemia or pernicious anemia) the altered hemoglobin (or hemoglobin content) modifies the osmotic resistance of the erythrocytes, and in these cases, the examination has a **diagnostic** value. In other cases, however, the test has only **theoretical** significance: on the one hand, it demonstrates that, like any other cell type, erythrocytes may also act as an **osmometer**, as they shrink or swell depending on the osmotic pressure of the suspending solution. In the latter case, first spherocytes are formed than the cell membrane ruptures resulting in hemolysis. On the other hand, the decreased osmotic resistance can be an **indicator** of the decreased resistance of red blood cell membrane against any impact.

Experimental procedure

100-100 μL of the diluted blood sample is added to 3 mL of various hypotonic NaCl solutions. NaCl concentrations of 0.9%, 0.8%, 0.7%, 0.65%, 0.6%, 0.55%, 0.5%, 0.45%, 0.4%, 0.3% and 0.2% should be used (all these concentrations are expressed in mass-volume percentage; g/100mL). Wait 2-3 min after mixing! Hemolysed samples will be red and transparent, whereas non-hemolysed ones remain red but opalescent**. Osmotic resistance is defined by the smallest NaCl concentration in which the integrity of the cell membrane remains preserved (i.e. no hemolysis occurs)**. The physiological value for osmotic resistance is 0.45-0.5 %.

More accurate results can be obtained if changes in the turbidity of the samples (indicating the development of hemolysis) are monitored using a photometer. In this case, the samples must be thoroughly shaken and the extinction at the wavelength of 540 nm should be monitored.

Repeat determination of the osmotic resistance in blood samples pretreated with either 75 or 100 mmol/L Thiocarbamide (the exact concentration will be specified by the tutor)! These concentrations of Thiocarbamide fail to evoke complete hemolysis, but they increase the volume of red blood cells resulting in decreased osmotic resistance. (**Note,** that this means, that hemolysis occurs in NaCl solutions of **higher** concentrations)

Dilute the blood sample 5-fold with isotonic Thiocarbamide containing saline (0.2 mL undiluted blood plus 0.8 mL Thiocarbamide solution). Add 100-100 μL of this solution to the series of hypotonic NaCl solutions (3 mL each), as described previously, in order to determine osmotic resistance of the Thiocarbamide-treated blood!

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2.1.5. Quantitative analysis of corpuscular elements in blood

The analysis may be performed manually by counting diluted blood in a Bürker hemocytometer (counting chamber) or using an automatic counter.

2.1.5.1. Counting in Bürker counter

Blood is first diluted (10-200 fold) with special solutions using Mélangeur (mixing) pipettes, then counted in the hemocytometer under a microscope.

2.1.5.2. Mélangeur (mixing) pipettes

These pipettes have polished tips, narrow stems graduated in tenth with figures showing 0.5 and 1.0. They then widen into a bulb containing red or white glass beads, used for counting the red blood cells (RBC) or white blood cells (WBC), respectively (Fig.**2.1.**). Finally, at the upper end of the pipette the bulb narrows again, and at this point it is marked 101 or 11 (depending on the type of the pipette). Thus the blood may be diluted to 10-20 fold prior to the WBC, and to 100-200 fold prior to the RBC count.

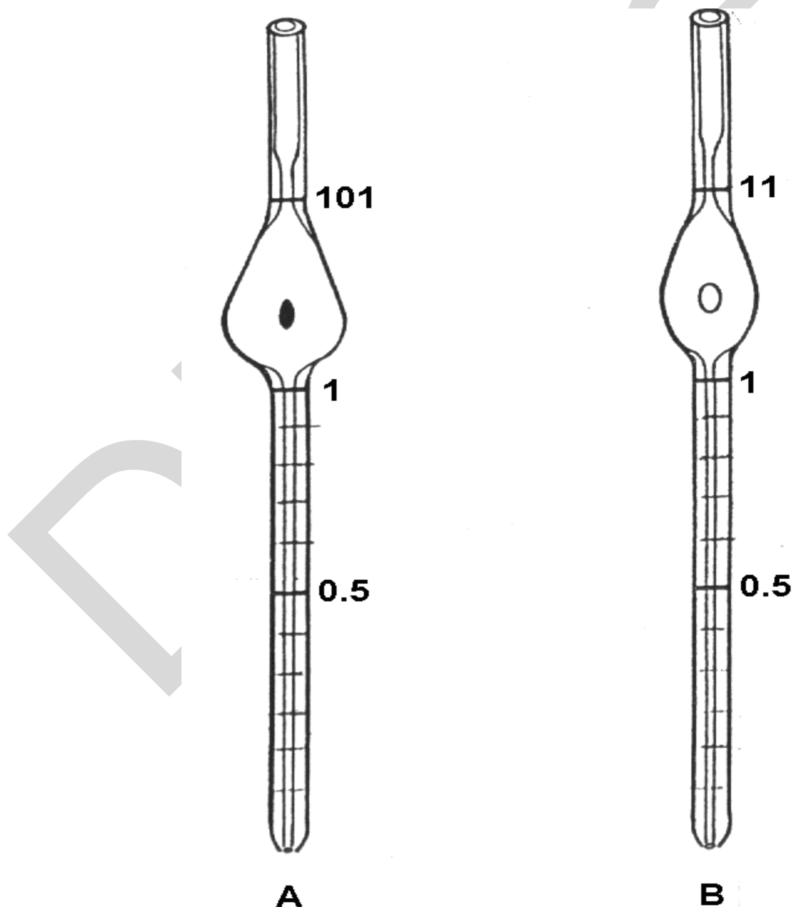

Fig.2.1.
Mélangeur pipettes used for dilution of blood for RBC (A), and for WBC counts (B)

2.1.5.3. Bürker counter

The Bürker counter is a thick glass or plastic slide with a central platform divided by a short transverse gutter into two parts . Arrows represent the areas to be filled with the diluted blood (Fig.**2.2.A**.), each ruled with a counting grid (Fig.**2.4.**). On each side of the platform, but separated by a trough, there is a support across the width of the slide, holding the special thick coverslip exactly 0.1 mm above the counting area (Fig.**2.2.B.**). The coverslip is fixed with two special clamps, covered with corkwood (Fig.**2.3.**).

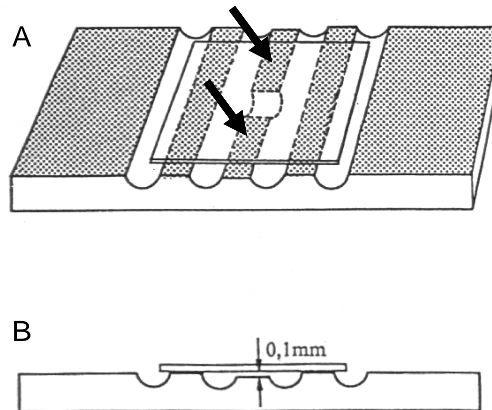

Fig.2.2.
Bürker counter with coverslip viewed from two aspects. The 0.1 mm height of liquid in the chamber is illustrated in panel **B**.

The hemocytometer and coverslip are ready to use, provided that they are properly cleaned, dried, and fastened (they should be free of grease or fibers). The fitting of the coverslip is correct when above both supports rainbow-colored concentric stripes (called 'Newton rings') can be seen when viewed from below. Their appearance proves that the distance between the two glass surfaces is less than the wavelength of the light; in this case, the space between the coverslip and the platform is exactly 0.1 mm (Fig.**2.2.B.**).

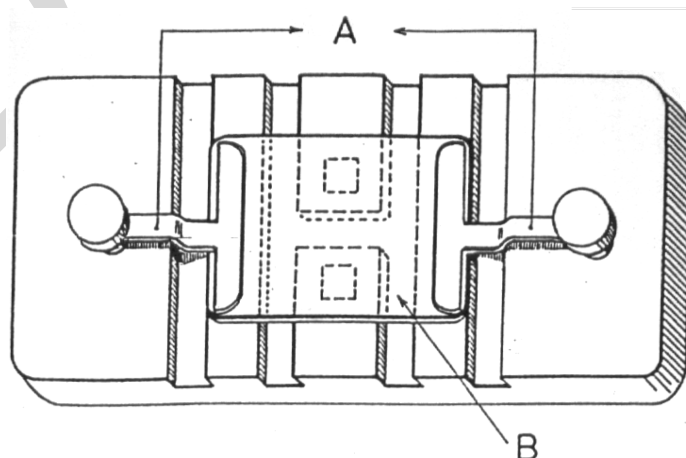

Fig.2.3.
Bürker hemocytometer with coverslip (B) and clamps (A)

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2.1.5.4. Solutions

Türk's solution is used for determining the **WBC count.** It contains: *acetic acid* (0.5 %) and a *dye* (1 % solution of *methylene blue* or *gentian violet*) to stain nuclei, improving visibility of the cells. The solution is hyposmotic in order to hemolyse red blood cells, which otherwise would disturb the WBC count (Note that disintegration of the WBCs also develops, but their stained nuclei are still present in the solution, and this is enough for the correct completion of the investigation).

Hayem's solution is used for **RBC count.** It contains 0.5 % NaCl, 2.5 % Na_2SO_4 and 0.05 % $HgCl_2$. Hayem's solution is hypertonic causing shrinkage of the red blood cells while preventing their aggregation.

2.1.5.5. Dilution of blood

The animal blood sample is diluted using the Mélangeur pipette with a mechanical pipette (Fig.**2.4.**).designed for this purpose. First attach the upper end of the mixing pipette to the mechanical pipette tightly but gently as the mixing pipette can break easily. Then suck the blood up to the **0.5 mark**, or slightly beyond it **without air bubbles**. Wipe off the blood adhering to its polished tip with your fingers (using medical gloves is highly recommended; note that tissue or cotton would steal blood from the pipette). If the blood is beyond the mark, tap the tip gently against the fingernail or palm of your hand until the blood is exactly at the mark. After this, draw a small bubble of air into the capillary.

Before starting dilution, make sure that the tip of the pipette is free of blood and the blood volume is correct. Suck up the proper solution to the mark above the bulb of the pipette (**mark 11 for WBC count** and **mark 101 for RBC count**), then pull off the mixing pipette from the mechanical pipette and shake the mixing pipette vigorously for 1-2 minutes to mix the blood with the diluting medium thoroughly. This is best accomplished by closing the ends of the pipette with your thumb and index or middle finger. After the sample is thorough mixed, cells will fail to sediment, thus the filled pipette may be stored in a horizontal position. The pipette stem contains pure dilution medium without blood, therefore, **the first couple of drops of suspension should be discarded**.

Fig.2.4.
Mechanical pipette with Mélangeur pipettes

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2.1.5.6. Filling the Bürker counter

After discarding the first couple of drops of suspension from the mixing pipette, dry the tip of the pipette. Take the prepared counter and touch the counting platform gently with the tip of the pipette just at the end of the coverslip. A small amount of the suspension will be drawn under the coverslip by surface tension. Blood suspension should not get into the central trough, because blood cells may accumulate in the trough and the count will not be accurate. The counting platform should be completely covered with the sample and should not contain air bubbles. Note that a greasy surface prevents proper filling.

2.1.5.7. Setting the microscope

Place and fix the hemocytometer on the stage of a microscope and focus the objective on the grid of the counting chamber. A magnification of 150 to 200-fold is optimal which can be obtained using a 10X ocular with a 15-20X objective. Narrowing the diaphragm of the iris can improve the visibility of the cells. Refocus the objective and start counting.

2.1.5.8. Cell counting

A grid is engraved into the counting platform covering an area of 9 mm^2 (Fig.**2.5.**). Triple lines indicate the limit of 1 mm distance, therefore, the size of one shaded area in panel **A** of Fig.**2.5.** is exactly 1 mm^2. The 9 mm^2 of total counting area grid contains 144 counting units (one of these is enlarged in panel **B** of Fig.**2.5.**).

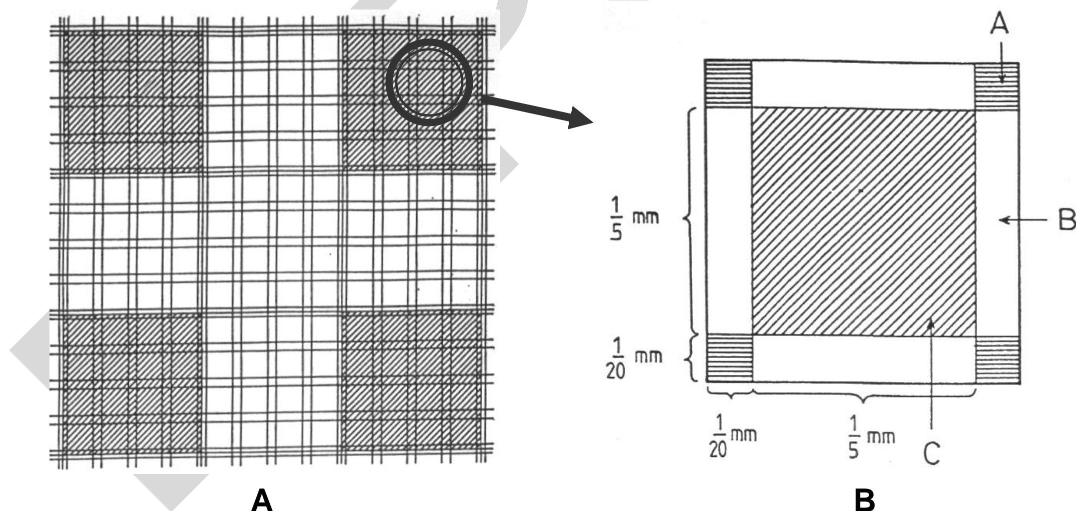

Fig.2.5.

Counting area with grid engraved into the counting platform of the Bürker counter.

Each counting unit contains a '**large square**' (area C), '**small squares**' (area A) and '**rectangles**' (area B). The area of a 'large square' is 1/5 mm x 1/5 mm = 1/25 mm^2, whereas that of a 'small square' is 1/20 mm x 1/20 mm = 1/400 mm^2, and that of a 'rectangle' is 1/5 mm x 1/20 mm = 1/100 mm^2. WBC count is performed conventionally on four 1 mm^2 surfaces (shaded in Fig.**2.5.A.**). RBC count is usually carried out using 'small squares'. Of course, any part of the area with grid may be used for counting, provided its dimensions are known. It is an important rule that cells

lying on 2 of the 4 boundary lines (for example North and East) must be systematically excluded when counting.

Calculation

Both the e**xtent of dilution** (in the Melangeur pipettes) and the **volume of the counting area** used should be considered, keeping in mind that the **height of the fluid column** above each territory of the grid is uniformly **0.1 mm**, and that the extent of dilution is **20-** and **200-fold,** in the cases of the WBC and RBC counts, respectively, assuming that the blood was sucked up to the 0.5 mark in the pipettes.

1. WBC count:

The course of the calculation is the following:

Height of the fluid column:	0,1 mm
Area of 1 square (surrounded by triple lines):	1,0 mm^2
Area of 4 squares	4,0 mm^2
Volume of fluid above 4 squares:	0,4 µL
	(1 µL=1 mm^3)

Knowing the **number of leukocytes (n)** and the **volume of fluid,** the concentration referring to 1 liter of blood can easily be calculated as follows:

n pieces of leukocyte ⟶ in 0,4 µl diluted blood

x pieces of leukocyte ⟶ in 1 liter (10^6 µL) diluted blood

from this: $x = (n \times 10^6) / 0,4$

The WBC number is determined after multiplying the result obtained in the above calculation with the extent of dilution (10x or 20x); in this case the number obtained refers to the number of WBCs in one liter of blood. This value is usually expressed as giga/liter (1 G/L = 10^9 leukocyte/L).

The physiological value for WBC count in healthy adults is 4.8-10.8 G/L, but in newborns or young children it may be somewhat higher. Deviation more than 15% is considered pathological.

2. RBC count:

Counting 20-20 'small squares' in each platform (altogether 40 squares) is recommended for the correct RBC count (area A in Fig.**2.6.B.**) The calculation procedure is effectively the same as in the case of WBC count, but the volume of diluted blood above these squares is altogether 0.01 µL.

Height of the fluid column: 0,1 mm

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Area of 1 small square : 1/400 mm^2
Area of 40 small squares: 0.1 mm^2
Volume of fluid above 40 squares: 0,01 µL
(1 µL=1 mm^3)

Knowing the **number of erythrocytes (n),** and the **volume of fluid** above 40 small squares, the red blood cell number in 1 liter of blood can easily be calculated as follows:

n pieces of erythrocytes ⟶ in 0,01 µL diluted blood

x pieces of erythrocytes ⟶ in 1 liter (10^6 µL) diluted blood

from this: $x = (n \times 10^6) / 0,01$

The RBC number is determined after multiplying the result obtained in the above calculation with the extent of dilution (100x or 200x); in this case the number obtained refers to the number of RBCs present in one liter of blood. This value is usually expressed as tera/liter (1 T/L = 10^{12} erythrocyte/L). The physiological value for RBC count is 4.7-6.1 T/L in healthy males, and 4.2-5.4 T/L in females.

The following **errors** may cause false results:

1. The diluting solution was previously contaminated with blood
2. The samples were not properly shaken and mixed
3. Wet or broken Mélangeur pipette was used
4. The blood contained bubbles when measuring
5. Only the diluting solution was loaded into the measuring chamber from the pipette stem instead of the properly mixed suspension
6. Loose coverslip, inaccurate volume in the counting chamber
7. Inhomogeneous distribution of the cells on the counting surface
8. Mistakes in calculation

It should be noted that the results obtained by this manual procedure are fairly badly reproducible and even skilled assistants may produce an average error of 10%. To deal with these uncertainties, hemoglobin concentration is often measured instead of the RBC count, or (if available) an electronic counter is used.

2.1.5.9. Electronic counting

Principles: Cells are poor electrical conductors due to the relatively high resistance of their cell membranes. If the diluted blood sample is pumped through a narrow glass capillary containing a pair of electrodes, changes in electrical resistance in the extremely thin stream of fluid will indicate the cells. In this way, proper filtering and setting of sensitivity in an impulse counter may count all types of corpuscles. For WBC count, RBCs have to be hemolysed. For counting thrombocytes, extremely pure diluting solution is necessary. Electric counters dilute blood automatically, therefore manual mistakes are prevented. Electronic counters require the use of

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venous blood. The special advantages of the electronic counting are the good reproducibility, accuracy, and rapidity.

2.1.6. Photometric measurement of hemoglobin concentration in blood

(Drabkin's method)

Theoretical background: The hemoglobin is released from the erythrocytes when the cells are treated with detergent (Sterox), then hemoglobin is converted to methemoglobin by $K_3Fe(CN)_6$ (Fe^{2+} is oxidized to Fe^{3+}). In the next step, methemoglobin is converted to cyanomethemoglobin using KCN. The absorbance of this compound, read on a spectrophotometer at 540 nm, is linearly proportional to the hemoglobin concentration of the sample.

Experimental procedure: 5 mL of Drabkin reagent (containing $K_3Fe(CN)_6$, KCN, KH_2PO_4 buffer and Sterox) is pipetted into a test tube. 20 µl (undiluted!) blood is added and properly mixed. After 5 min, the absorbance is measured in a spectrophotometer at 540 nm. Pure Drabkin reagent (without blood) should be used as blank. Although the properly executed procedure is harmless, as the KCN solution is well buffered, **one must be very careful, since the solution contains cyanide, which may be converted to the extremely poisonous HCN in the presence of acids. Application of mechanical pipettes is mandatory.** Drabkin reagent is light-sensitive, and it should be stored in a brown container and kept in dark.

Calculation: concentration of hemoglobin = A_{540} * 368 (g/L)

Evaluation: Hemoglobin concentration is 130-165 g/L in healthy males and 115-150 g/L in females.

Mean Corpuscular Hemoglobin (MCH) and RBC Index (RBCI)

The average hemoglobin content of a single RBC, called **mean corpuscular hemoglobin (MCH)**, can be calculated using the hemoglobin concentration and RBC count of the blood sample according to the following equation:

$$\text{MCH (pg)} = \frac{\text{Hemoglobin concentration (g/L)}}{\text{RBC count (T/L)}}$$

Normal value: 27-31 pg (1 pg = 10^{-12} g)

RBC index, (RBCI) is a relative number, which indicates the relative hemoglobin content of a single erythrocyte in the investigated blood sample compared to the supposedly normal hemoglobin content of a normal erythrocyte.

Calculation:

$Hb_{Actual} / Hb_{Normal}$

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$$RBCI = \frac{\qquad}{RBC\ count_{Actual} / RBC\ count_{Normal}}$$

Evaluation: RBCI = 1 → normochrome RBCs

RBCI < 1 → hypochrome RBCs

RBCI > 1 → hyperchrome RBCs

Example for the calculation

In the test sample, Hb = 75 g/L and the RBC count = 2 T/L. Considering Hb = 150 g/L, and RBC count = 5 T/L, as physiological values (Normal), the calculation is as follows:

$$RBCI = (75/150) / (2/5) = 0.5 / 0.4 = 1.25$$

In this blood, both the RBC count and Hb are reduced, however, the RBC count was more significantly decreased than the Hb concentration. Consequently, these erythrocytes contain more hemoglobin than under physiological circumstances. Pernicious anemia is characterized by markedly increased RBCI, thus the RBCs are hyperchromic. Reduced iron absorption results in hypochromic anemia with strongly reduced Hb. During bleeding (for whatever reason; e.g. menstruation, ulcus, etc.), whole red blood cells are lost, thus the Hb concentration and the RBC count reduce proportionally, resulting in normochromic anemia.

2.1.7. Hematocrit and Mean Corpuscular Volume (MCV)

Hematocrit

Hematocrit indicates the proportion of the total volume of blood cells in a unitary volume of blood. The hematocrit value may be expressed as a percentage value (i.e. 45%) or as a portion (i.e. 0.45). Both cases mean that out of 1 L of whole blood, 450 mL is provided by the cumulative volume of the corpuscular elements (almost exclusively by the erythrocytes), whereas 550 mL is the volume of plasma.

Determination: The blood cells are separated from the plasma by centrifugation. The tube used in this test is a capillary hematocrit tube filled with venous blood up to 60-70 % of its length. The wall of the tube is coated with anticoagulant (usually heparin). When kept horizontally, the blood fills the tube spontaneously due to capillarity. In practice, shake the original blood sample well and put a few drops onto a hourglass, and fill the capillaries from this blood. As approximately two thirds of the tube is filled up, its lower end is blocked by a plastic plug. The tube is then centrifuged between 10,000 and 20,000 G for 5 min to separate the cellular elements from the plasma. The height of the packed cells in the tube indicates the hematocrit.

Evaluation: Since the height of fluid in the tube is variable, a special scale is used for evaluation. The bottom line of the blood in the capillary is aligned to the 0 (0%) line of the scale. Then shift the tube along the scale until the top of the fluid

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matches the 1 (100%) line. The position of the top of the RBC column can be read from the scale (Fig.**2.6.**). The physiological value is 0,39-0,50 (39-50%) for males, and 0,35-0,47 (35-47%) for females.

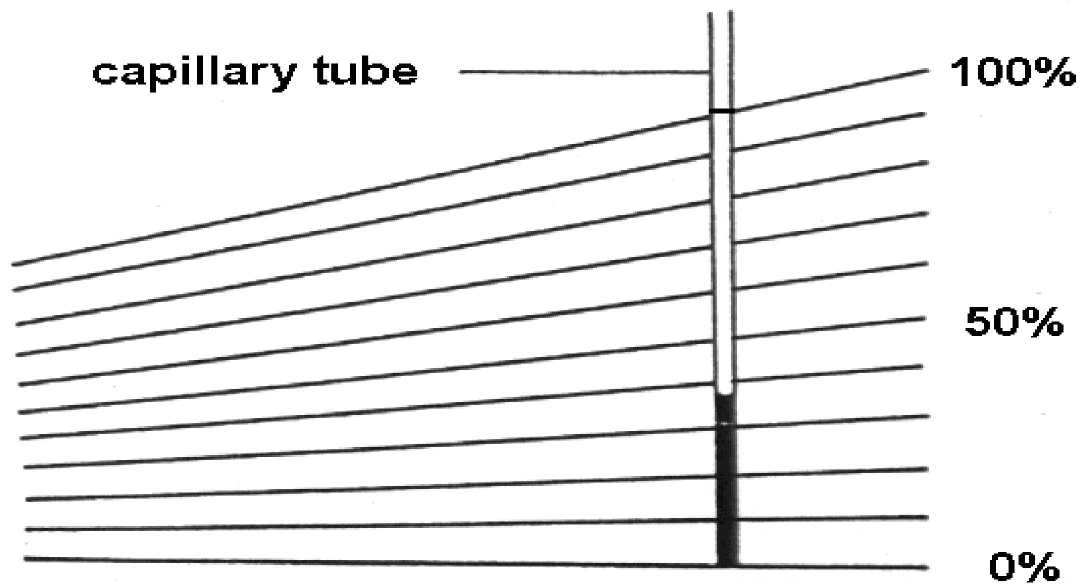

Fig.2.6.
Determination of the hematocrit

The most frequent sources of **error** are:

1. Crystalline salts as anticoagulants (oxalate or citrate) may shrink erythrocytes causing an underscore of the packed cell volume
2. Using capillary blood, contamination with interstitial fluid may cause underscoring
3. Inappropriate mixing of blood with anticoagulants
4. Too short centrifugation period

Calculation of the Mean Corpuscular Volume (MCV)

MCV represents the average size of a single erythrocyte, calculated on the basis of the following formula:

MCV (L) = hematocrit / RBC count

During the calculation one should keep in mind that in order to obtain the correct result, the hematocrit value must be expressed with a number between 0 and 1 instead of a percentage value (e.g. 50% = 0.5) and the RBC count should be used in number of cells/L! The result yields a number in liter, which when multiplied with 10^{15} gives a value in femtoliter (1 femtoliter = 10^{-15} liter). The physiological value of MCV is usually between 80 and 99 fL. According to this, the cells may be normocytic, microcytic or macrocytic. MCV represents an average figure, and does not give information about the size distribution of the erythrocyte population.

2.2. Experimental conditions

Experiments in the physiological practice are carried out on isolated preparations obtained from **mammals.** During the preparation procedure one of the most important things is to avoid mechanical damage (ravage or overstretch) and drying. Due to concomitant cell damage, these will increase the K^+ concentration in the extracellular space of the tissue, resulting in depolarization of the still intact cells, and consequently, in abnormal function. These preparations preserve their function only in a bathing solution having physiological ionic composition, osmotic pressure, constant temperature and reasonable oxygen and glucose environment. In these cases, Tyrode solution is used in the laboratory practice with continuous oxygenation.

Composition of Tyrode solution:

NaCl	144.0 mmol/L
KCl	5.5 mmol/L
$CaCl_2$	2.5 mmol/L
$MgCl_2$	1.2 mmol/L
Glucose	8.3 mmol/L
Tris-HCl buffer	5.0 mmol/L
Osmolarity	300 mosmol/L
pH	7.4

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2.2.1. Recording the mechanical response of muscle

The actual value of muscle strain can be measured by an electronic strain gauge, called transducer. This device is used when the effects of electrical stimuli and effects of chemical agents or drugs are studied. The instrument consists of a measuring head (detector) and an amplifier. The detector has a displacement to a voltage converter, and its output voltage is proportional to the displacement of the measuring arm from the unloaded, zero position. The mechanical and geometrical parameters have been designed for an **isometric** mode, therefore, the traveling distance of the measuring arm is negligible compared to the length of the muscles usually measured. (Isometric recording mode: measuring the strain of the muscle while its length remains constant.) The output signal of the detector is amplified by the amplifier to the required level (1 V for full-scale deflection) for the strip chart recorder. The amplifier gain is adjustable with a rotary switch (1X, 2X, 5X, 10X, 20X positions) and a two-position slide switch (1X or 10X positions) yielding a final amplification of 1 to 200. Carefully select the appropriate gain, since the application of too small gain makes the effect invisible, whereas too high gain causes an out of range value, hence the tension could not be measured.

The correct baseline of the transducer-amplifier system is essential for the proportionality of the recorded signal. The output signal of the transducer can be

visualized and recorded after digitalization on the computer. At any given setting of the system, the device has to be calibrated using a series of small weights in order to determine the calibration factor for the given experiment. This calibration should be linear - by theoretical consideration - therefore, 4 to 5 calibration points are sufficient. One must be sure that the highest value measured is below the highest calibration point.

2.2.2. Procedure of the measurement

1.: Tie the thread fixed to the tendon of the muscle to the end of the measuring arm.

2.: Using the lifting screw of the transducer, adjust the appropriate stretch of the muscle. (Try to adjust the length of muscle to be equal to the length before dissection.) This tension later will be called as **resting tension**, and the length as **resting length**. At this point, the measuring bridge has to be balanced using the rotary switch and potentiometer labeled **balance**. The transducer is balanced when neither of the imbalance LED's is on. **This balance setting should not be modified until the end of the given recording**.

3.: Carry out the prescribed chemical treatment or electrical stimulation in order to find out the correct settings of the measuring setup. Adjust the setting accordingly.

4.: Carry out the prescribed chemical treatment or electrical stimulation and record the response using a computer or strip chart recorder or an oscilloscope.

5.: Without changing the settings of the instrument, calibrate the system using the series of weights applied to the transducer system in the following way.

5.1.: Detach the muscle thread from the measuring arm.

5.2.: Set the correct balance of the amplifier.

5.3.: Using 4-5 calibration weights, calibrate the device recording the corresponding response of each weight combination.

5.4.: Using the weight-deflection pairs obtained above construct the calibration curve.

5.5.: Based on the calibration curve, calculate the appropriate strain (tension) values of the muscle for the required points.

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2.3. Computer operated Data Acquisition System

2.3.1. Measuring analog data using microcomputers

Processes occurring in nature are described with functions that are continuous in respect to time (analog). In the world of computers, on the other hand, the information is stored as discrete numbers. The basic unit of information is the bit that has two values: yes = 1 or no = 0. When a computer is employed to measure external, analog signals, first the conversion of these analog signals to discrete numbers (to digital numbers) should take place (analog-digital conversion). Without discussing the technical details, we would like to emphasize that all converters need the minimal and maximal measurable values (input range) to be set before the conversion can take place. In order to follow the process in time, one has to tell the computer how frequent the analog-digital conversion should be; i.e., what is the acquisition frequency (measured in Hz; 1 Hz = 1 point/second). It follows that an incorrect input range or acquisition frequency can render the measurement unusable.

2.3.2. Data acquisition and processing

All programs performing data acquisition try to be as user friendly as possible and will thus, on the one hand, constantly remind the user of the status of the measurement and, on the other hand, set up easy ways to control the parameters of acquisition. Therefore, the program can be instructed using hot-keys or via menus (these can be read directly from the screen, or the HELP function of the program will give detailed information on all possible commands). Furthermore, the measured signal is continuously displayed.

The reason why computer aided data acquisition and/or control has spread so rapidly to all areas is because the measured signal can be stored for later analysis and the process can be controlled as it progresses. The data acquisition program enables the user to store and then read the measured data and to apply current pulses during the measurement.

Once a set of data is stored it can be analyzed or printed. These functions, in a simplified way, are also parts of the acquisition program. However, if detailed analyses or special presentation is required, using graphs and/or tables, specific software should be used.

2.3.3. The data acquisition program

The computer operated data acquisition system provides the facilities offered by an electrical stimulator combined with an oscilloscope and a recorder. According to this, the applied software can perform and control the following parts:

- generation of electrical stimuli
- data acquisition, displaying the data on the screen
- storage of data

The data acquisition program can be started by the MEASURE icon. The program is controlled using buttons appearing on the screen and activated by clicking with the

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mouse. The current parameters of stimulation and data acquisition can be set by the user on the screen by typing or using the up and down arrows. The modified parameters will be valid by the next measurement.

2.3.4. Stimulus protocol

The software generates square wave pulses. Characteristic parameters of these square pulses can be defined using the buttons in the right side of the screen. Activation of the PULSE button results in delivering the pulse with the defined frequency. The second pressing of PULSE button switches off the stimulation. The defined stimulation is visualized on the screen.

2.3.5. Data acquisition

The duration of sampling can be preset using the DURATION button. When studying phenomena occurring rapidly (such as are contractions of skeletal or cardiac muscle), a short (60 second) sampling duration is to be chosen. Slowly developing phenomena, like mechanical activity of smooth muscle, require longer (10 min) sampling durations. By increasing the duration the frequency of the sampling should be decrease because the acquired data must fit into the memory of the computer.

The actual measurement (i.e. sampling) can be initiated by clicking the **START measurement** or terminated using the **STOP measurement** button. During measurement the acquired data is visualized on the left side of the screen.

Although the system is able to handle two preparations simultaneously, only one preparation will be associated to each system during the practical. Sampling of the second channel is associated to the stimulus or the MARKERs. Amplification of the acquired channel can be adjusted by predefining the sampling interval.

2.3.6. Storage of data

Recorded data can be saved at the end or the interruption of a measurement. The software automatically offers a data directory. Please use only letters and numbers (without Space and any extra characters) in the file name. It is reasonable to write this file name into the exercise book, this may help to identify the respective record later during data analysis or printing.

Do not forget to store your data before analyzing it. In case you forget to save your record, the next start of data acquisition will delete the data from the memory and you have to repeat your measurement.

2.3.7. Data analysis

You can start the data analysis by the **ANALYSIS** icon. The function of the program can be activated by clicking with the mouse on the appropriate button.

Before starting the analysis, the appropriate record must be opened and using the left buttons of the mouse the left (green) and right (red) cursor must be placed.

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The program calculates automatically the following parameters (see Fig. **2.7.**): maximal amplitude (Amp), slope, integral, time to peak value (TTP), half-relaxation time (HRT). You can find the time (x) and voltage (y) values of the cursors and they distance (Δx, Δy). By using the Δx values the average cycle length (CL) of the contractions can be calculated. In case you know the calibration constant of the force transducer used during the measurement, you can set it in the program. Note that although the conversion is automatically carried out by the program, the title of the Y-axis remains V (Volt).

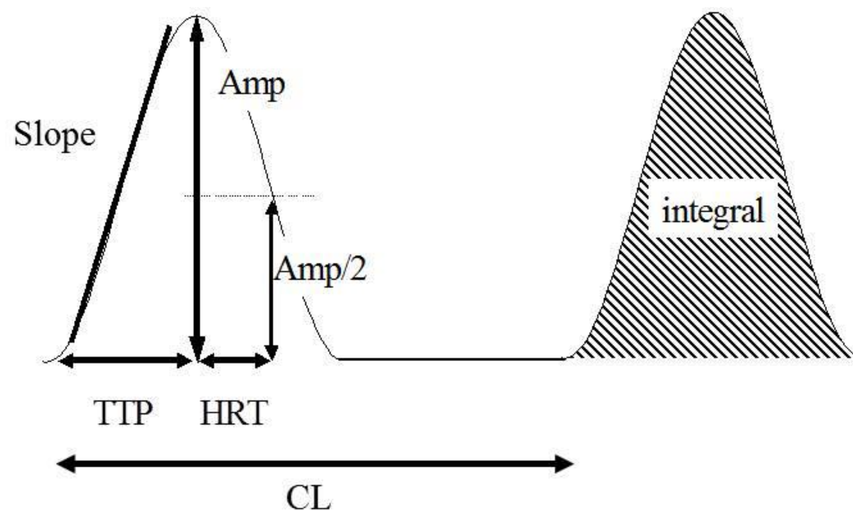
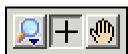


Fig. 2.7.
Parameters of the automatic analysis

 With the graph palette, you can move cursors, zoom, and pan the display. The graph palette appears with the following buttons, in order from left to right:

- **Zoom**—Zooms in and out of the display. Use the pull-down menu that appears when you click this button to select a zoom method.
- **Cursor Movement Tool**—Moves cursors on the display.
- **Panning Tool**—Picks up the plot and moves it around on the display.

Click a button on the graph palette to enable zooming, moving cursors, or panning.

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2.3.8. Printouts

You can select a portion of the record for printing by the cursors. The left bottom graph will show the picture to be printed. If the picture is right then define its name and click the **Save picture** button.

To print the saved pictures please use the inbuilt program of the operation system. Open the **Windows Intéző** from the **Taskbar** and find your pictures on the local hard drive (Számítógép, Helyi lemez C:) in the **Gyakorlat** directory. Using the **CTRL** key and the mouse select the pictures for printing then open **Nyomtatás** in the top menu. Four selected pictures can be organized by choosing **9 x 13 cm (4)** option

on **landscape page (**fekvő lap). Please switch off Picture fill to edges (**Kép töltse ki a keretet)** function. Set the number of necessary samples and start printing. Printing is relatively slow; therefore, it is reasonable to print all the selected files together, after finishing the work.

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2.4. Experiments on smooth muscle

2.4.1. Experiments on uterine smooth muscle of the rat

2.4.1.1. Theoretical background

Smooth muscles are more sensitive to humoral factors (neurotransmitters, endocrine, paracrine and autocrine substances) than either cardiac or skeletal muscle. In addition, the myometrium undergoes hormonal changes according to the menstrual cycle and pregnancy. Changes in the sensitivity to humoral agents are dependent on changes of cellular membrane receptor composition and sensitivity, which together determine the coordination of transmembrane signalization. The transmembrane signalization itself can be studied by the application of different agonists and antagonists.

The smooth muscle function requires the presence of different ions in the extracellular space. Both reduced extracellular calcium concentration and increased magnesium level reduce the spontaneous activity of the myometrium; the latter effect is the consequence of the inhibition of the voltage-gated Ca^{2+} channels. Barium causes sustained contraction of smooth muscles. This ion reduces the potassium conductance of the membrane, and thereby causes depolarization and prolongs or makes repolarization impossible.

Agents that increase the mechanical activity of the myometrium are called **uterotonics**. Prostaglandin, oxytocin and acetylcholine are the major representatives of this group. Acetylcholine acts on muscarinic receptors, its action is, therefore, inhibited by atropine. All the uterotonics mentioned above are natural components of the body. They are physiological regulators of uterine smooth muscle.

Similarly, physiological regulators of uterine activity are catecholamines as neurotransmitters, like epinephrine (Tonogen). Epinephrine induces hyperpolarization of smooth muscle cells, and it decreases the rate of spontaneous rhythmic contractions as well as the tone of the myometrium. Epinephrine acts via β_2-adrenergic receptors. Pindolol is a non-specific beta-receptor blocker, and is therefore able to antagonize action of epinephrine on the β_2-receptors.

Both the α- and H_1-receptor agonists (phenylephrine and histamine, respectively) evoke smooth muscle contraction by increasing the intracellular calcium concentration. The effect of phenylephrine can be antagonized by phentolamine (an α-receptor blocker).

Papaverine directly relaxes all smooth muscle cells, partly by inhibiting cyclic nucleotide phosphodiesterase and thus increasing the intracellular cAMP concentration.

It must be noted that although the effects of neurotransmitters are easy to demonstrate, their physiological effects are not principally important; in fact, they are rather secondary in controlling the function of the myometrium. Moreover, since their actions develop only at higher than physiological concentrations, these actions are regarded as pharmacological effects.

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Experimental protocols

2.4.1.2. Effects of neurotransmitters, their agonists and antagonists

2.4.1.2.1. Effects of epinephrine

50 µL of **epinephrine (Tonogen)** solution is added to the organ bath of the myometrium showing normal, rhythmic contractions. After 10 minutes, 50 µL of **pindolol** solution is added, and 20 minutes further recording is recommended. After this period of time, the bathing solution must be replaced by drug-free Tyrode solution. Washing the preparation for another 20 minutes will prove the reversibility of the previous effects.

2.4.1.2.2. Effects of acetylcholine

100 µL of **acetylcholine** solution (having concentration of 10 µg/mL) is added to the tissue bath of the myometrium showing control mechanical activity. Follow the contractions for 10 to 30 minutes, then return to drug-free Tyrode solution. Wash the preparation several times until the control pattern of activity returns. Repeat the same acetylcholine-treatment in the presence of 50 µL of **Atropine** injection (the muscle has to be preincubated with atropine for 10 minutes). Atropine alone is not expected to influence the mechanical activity of the uterus; however, it is able to prevent the effect of the second acetylcholine treatment. After the contractility is recorded for further 20 minutes, wash the myometrium with Tyrode solution until its activity normalizes.

2.4.1.2.3. Effects of phenylephrine

Phenylephrine is a selective α-receptor agonist, whereas **phentolamine** is a selective α blocker.

Apply 40 µL of **phenylephrine** solution (10 mM stock solution of phenylephrine). Record the effect of phenylephrine on the uterinal contractions, then add 50 µL of **phentolamine** solution to the bath, and continue recording until the effect of phenylephrine decreases. Finally, return to drug-free Tyrode solution, and record the reversal of the effects.

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2.4.1.3. Effects of uterotonic drugs

2.4.1.3.1. Effect of histamine

Add 40 μL of **histamine** solution (10 mM stock solution) to the tissue bath of myometrium showing normal mechanical activity. Record the effect of histamine and when the muscle relaxes repeat the histamine treatment (40 μL). Compare the duration of smooth muscle contractions elicited by the first and second histamine treatment.

2.4.1.3.2. Effect of oxytocin

100 μL of **Oxytocin** injection is added to the myometrium showing normal mechanical activity. After recording the effect of oxytocin for 10-15 min, add 20 μL of **papaverine** solution (Papaverine hydrochloricum ampoule) to the bath, and carry on recording the effect of papaverine on the uterinal contraction.

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2.5. Studying the function of peripheral nerves and the innervated muscles

2.5.1. The compound action potential

Compound action potentials can be recorded using extracellular electrodes on nerves consisting of numerous axons (Fig.**2.8.**). These action potentials are generated from the summation of the action potentials of the individual axons. As such, the size of the potential change (e.g. the area under the curve) is proportional to the number of axons creating the potential change. If the site of electrical stimulation is located substantially far away from the recording electrodes, the compound action potential consists of more waves on the record due to the difference between the conduction velocity of the individual types of axons. During the lab, we record a compound action potential of a nerve fiber containing 5 different types of axons. Knowing the electrode distance (s), the time between the peak of the individual waves, and the point of stimulation (t), we can calculate the conduction velocity of each type of axon. The larger the diameter of an axon and the thicker its myelin sheet, the faster its conduction.

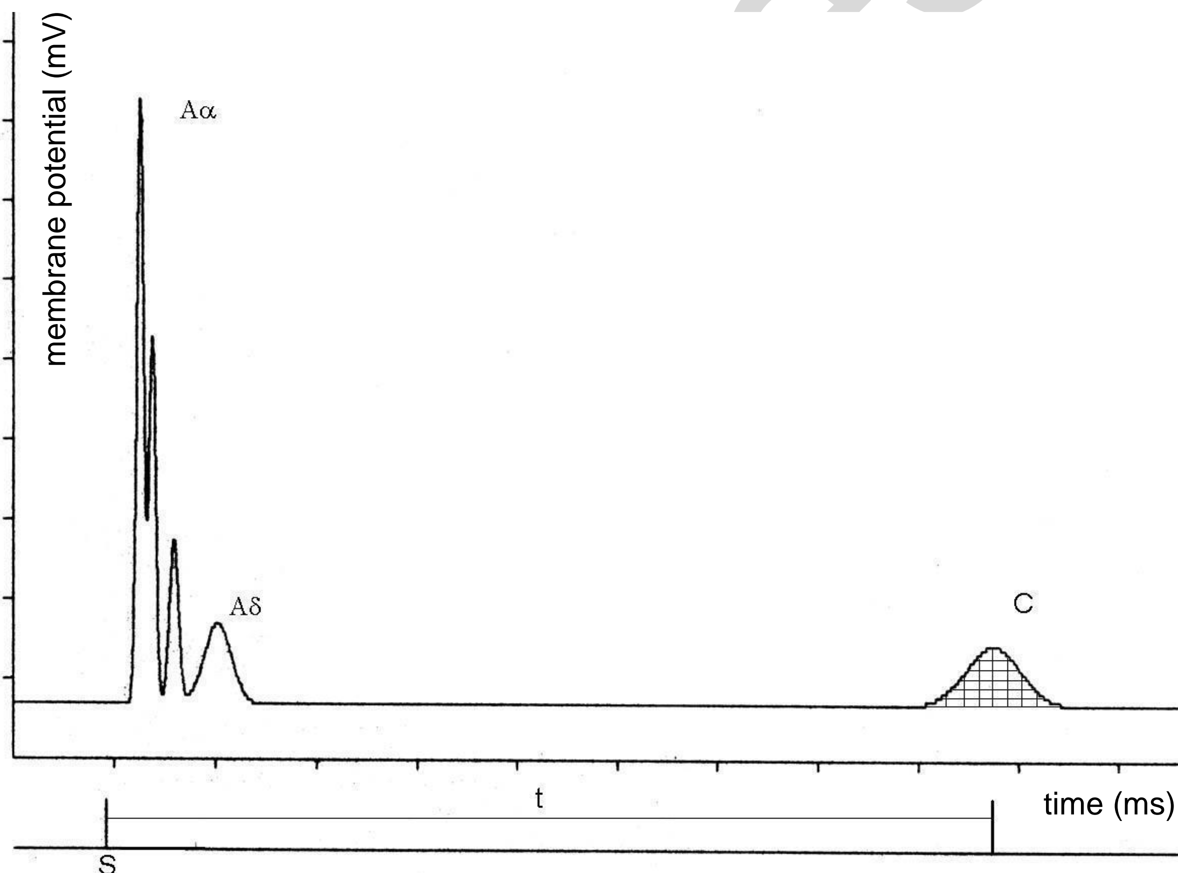

Fig.2.8.

A compound action potential. The peaks of the different types of axons and the time of stimulation (S) are indicated. The area under the curve (for example see type C fibers) is proportional to the number of axons belonging to that particular type. „t" indicates the conduction time of the type C fibers.

2.5.2. Receptor potentials

The information originating from the environment and eventually acting upon the nervous system always evokes a change of the resting membrane potential of the sensory receptor cells first. This membrane potential change is always an electrotonic potential, which is called a receptor potential. Although the amplitude of the receptor potential depends on the strength of the stimulus, there is a maximal value that cannot be exceeded even if very intense stimulation is applied. If the receptor potential is large enough, it will induce a single or a series of action potentials. If the stimulus intensity and the concomitant receptor potential increase, the frequency of the action potentials increases as well. In other words, the sensory neuron is transmitting the strength of the stimulus in a frequency-encoded manner.

When the stimulation is maintained for a reasonably long period of time at constant intensity, the receptor potential of the slowly adapting (tonic) receptors (and the frequency of the action potential firing) decreases only to a small extent. In the rapidly adapting (phasic) receptors, however, the amplitude of the receptor potential (and the frequency of the action potentials) decreases rapidly, and both may be completely abolished during long lasting stimulation. In the cases of the slowly/rapidly adapting (tonic/phasic) receptors, the amplitude of the receptor potential first decreases rapidly, then this decrease stops at a level where action potentials are still generated, although at a lower frequency than at the beginning of the stimulation.

During the lab, receptor potentials of the slowly/rapidly adapting sensory cells are registered by intracellular microelectrodes. Later, action potentials trains are recorded from an axon during prolonged stimulation. In the rapidly adapting receptor, despite the maintained stimulation, the receptor potential quickly diminishes, and the action potential firing is rapidly terminated. On the other hand, during prolonged stimulation of a slowly adapting receptor, the reduction of the receptor potential is not prominent, and action potentials can be recorded from the appropriate axons during the entire length of the stimulation. Although there is a change of the firing frequency, but the reduction is not significant, and it occurs slowly, especially towards the end of the stimulation.

2.5.3. Smooth muscle action potential

When the slow membrane potential changes (slow waves) of the smooth muscle are generated, first inward calcium then outward potassium currents are activated, causing depolarization and repolarization of the membrane, respectively. In certain cases, when the most positive membrane potentials are reached during the slow wave activity, action potentials may also develop. The amount of calcium entering the cell during these action potentials is sufficient to increase the intracellular calcium concentration and initiate contraction.

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2.5.4. Tetanus evoked on slow and fast muscles

Muscles are composed of different types of muscle fibers. It must be noted that not only the ATPase activity of the myosin molecules of these fibers is different, but their metabolic properties and contractile parameters are also rather diverse. The muscles, which contain more slow type (I) fibers are called red muscles. These muscles respond slowly to stimulations, their latency is long and they are adapted to long-lasting, slow contractions. White muscles, on the other hand, containing mostly type IIB fibers are fast responders and can perform rapid, powerful and precise movements. The difference of the various types of muscle fibers is mostly the consequence of the difference of the contractile proteins: in type I fibers the ATPase activity of the myosin isoenzyme is slow, whereas it is quite fast in type II fibers. Considering the basic properties of the individual types of skeletal muscle fibers, high frequency excitation causes a slowly developing tetanus in the slow muscles, and fatigue is not apparent during the entire length of the stimulus. In the fast muscles, however, tetanus appears rapidly, and after reaching the maximal contraction, the force generated rapidly decreases as the consequence of fatigue.

During the practical tetanic contraction is registered from slow and fast muscles. Students should determine the time to peak and the maximal contraction of the mechanical responses, both at the beginning and at the end of the stimulation.

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3. COMPUTER SIMULATIONS

3.0.1. General introduction to the computer simulations

Computer simulation programs, through their mathematical approach to studying different physiological processes, enable one to understand the major characteristics of the investigated processes, demonstrate the relationship between different parameters and emphasize the practical use of the studied principles. Their importance is not merely the visual presentation of a given process but they also provide quantitative insight into the operation of various physiological systems without any advanced mathematical knowledge.

In order to bring simulations as close to reality as possible, a number of parameters have to be included into the actual model. It should be noted that on the one hand, most of the programs can simulate processes that are not parts of the 'Physiology Practice'; and on the other hand, even the simple description of the possibilities is far beyond the limits of this 'Laboratory Guide'.

Each of the simulations is presented in the 'Laboratory Guide' separately, with enough information to understand the basic principles. The mathematical equations forming the **theoretical background** of the simulations are presented as condensed as possible and, where appropriate, the equations were repeated in the text. When discussing a given simulation, the **structure of the program** together with the actual **simulation screen** is described in detail. Finally, a short section will give some **aspects** on how to **evaluate** the curves presented by the program. Reading alone will not replace the practice, a **program can only be understood** in front of the computer **when the simulation is in use**.

As all programs designed for public use, these simulations have several characteristics in common. They all contain an introductory part that gives details on how to run the program and what the basic principles used to construct the simulation are. The majority of the programs contain demonstrations, in which cases the simulations are run with preset parameters.

The programs also provide the possibility to enter user-defined data, thus they enable studying the effects of the various altered parameters. The 'Exercise Book' contains all necessary information to show how to use these parts of the programs, and how to describe the results. It is very important to indicate every detail that helps understanding the graphs (e.g.: using different colors, application of labels, etc.) when copying the results from the screen.

3.0.2. Using the simulations

After starting the computer it either asks for a PASSWORD (in the case of older machines) or you have to choose the STUDENT login name (with new computers). In the new computers the simulations (except skeletal-muscle function simulation) can be started by clicking on the icon MANAGER icon on the desktop. Skeletal-muscle function simulation can be started by clicking on the icon PhILS on the desktop. In the older PCs you have to type STUDENT to start the network connection! STUDENT must be used for the LOG ON Name and for the

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PASSWORD, too. After pressing ENTER, the MENU program called PROGRAM MANAGER will automatically start and offer choices to run the simulations in Hungarian or in English. (If for some reason it does not start, you can start it just as with the new PCs by clicking on the MANAGER icon on the desktop.) When the 'working language' has been selected by the TAB key, you can run the different programs using the cursor movement arrows and pressing ENTER. To proceed in a running program (or scroll in the information part) follow the instructions in the given program, or in the absence of any indication, press any key.

Be careful to respect the limits of the parameters given in the programs during user-defined data entry. Data entry is confirmed by pressing ENTER. Defective data will not be accepted by some of the simulations and are automatically deleted; others will remind you that the appropriate limit was exceeded. The computer program will usually not run unless correct data are entered. In case of defective data input, the simulation might not work satisfactorily or it may stop; in this case let the tutor know that this happened.

To exit from any given program, follow the instructions on the screen. After exiting, the list of the available simulations appears again and a new program can be selected.

Due to the fact that the computers are part of a network, some commands executed from the DOS prompt will affect other parts of the system and hence cause quite annoying problems. Therefore, **please** do not use computers for anything but the task set by the tutor or the practice schedule.

3.0.3. Studying the characteristics of ligand-receptor interaction

Receptors are macromolecules capable of binding certain ligands. Receptors have one or more active centers. The binding of the ligand to (a) specific active center(s) results in a physiological response. The receptor might have an allosteric center as well; binding to this center does not induce any response but can either enhance or attenuate the effect of the specific ligand. Ligands, in general, can be agonists or antagonists.

An agonist is a substance that can bind to the same binding site and will induce the same response as the natural (usually endogenous ligand) of the receptor. A full agonist is a ligand that will induce the maximal possible biological response. Partial agonists have smaller efficacy (see later) and will induce smaller responses even if binding to all receptors.

From the pharmacological point of view, an antagonist is a substance that will bind to the same receptor as the agonist, but will decrease or prevent the effect of the latter. A clear pharmacological antagonist will bind to the specific or allosteric center of the receptor and will prevent the agonist from exerting its effect without inducing an effect of its own. This type of antagonism can be either reversible or irreversible. If raising the concentration of the agonist removes the antagonist from its binding site, the binding is called reversible antagonism. If the binding of the antagonist is so strong (e.g. covalent bound) that increasing the concentration of the agonist has no effect, the binding is referred to as irreversible antagonism. Competitive antagonism

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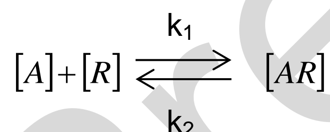
is a form of reversible antagonism, when the agonist and antagonist compete for the same binding site. If the concentration of the two substances are the same, the binding site will bind the molecule that has the higher affinity (see later). Raising the concentration of the competing partner will displace the other compound from the binding site of the receptor. On the other hand, a non-competitive antagonist will bind to a part on the receptor to which, under physiological conditions, the agonist will not.

The ligand-receptor interaction depends on the size, conformation and electric charge of the molecules. The magnitude of the effect is determined by the concentration of the ligand, the number of occupied receptors (set by the affinity) and the number of receptors available.

Dissociation constant, half effective concentration, half inhibitory concentration

The following parameters are used to describe the ligand binding: dissociation constant (K_d), half-effective concentration (EC_{50}), half-inhibitory concentration (IC_{50}), affinity, potency and efficacy.

For reactions that reach equilibrium, the rate of appearance of the product (AR) formed from the starting compounds (A=agonist and R=receptors) is constant:



$$[A]+[R] \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [AR]$$

where k_1 is the rate constant of association, whereas k_2 is the rate constant of dissociation. (The followings will consider the simple case when there is only a single binding site on the receptor for the agonist.) The ratio of k_2 over k_1 gives the dissociation constant. EC_{50} will give that concentration of the ligand that produces half of the maximal possible response (Fig. **3.1.**).

The half-inhibitory concentration, IC_{50}, is the concentration of the antagonist which blocks 50% of the effect of the agonist (Fig. **3.2.**).

The affinity is a measure of how strong the binding of the ligand is to its receptor. The affinity is the reciprocal of the K_d.

Potency will give the amount of ligand necessary to reach a certain physiological response. From two ligands having the same effect ,the one that evokes the response at a lower concentration is the more potent. Potency depends on the K_d and the efficacy. The smaller the K_d, the greater the potency.

Efficacy shows the maximal attainable response, independent of the concentration used. Thus efficacy can only be expressed in relative units. This parameter is used to compare different agonists. If two ligands reach maximal effect at the same concentration, but these two maximal responses differ, the agonist giving rise to a larger response has greater efficacy. A partial agonist is said to have an efficacy of 1 if the maximal response it can evoke is the half of the actual maximum characteristic for the given tissue. A full agonist will have an efficacy greater than 1, while a clear competitive agonist has an efficacy equal to 0.

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Analyzing dose-response curves

To describe the quantitative aspects of the ligand-receptor interaction, dose-response curves are constructed. The effect, expressed as percentage of the attainable maximum, is plotted as the function of the agonist concentration.

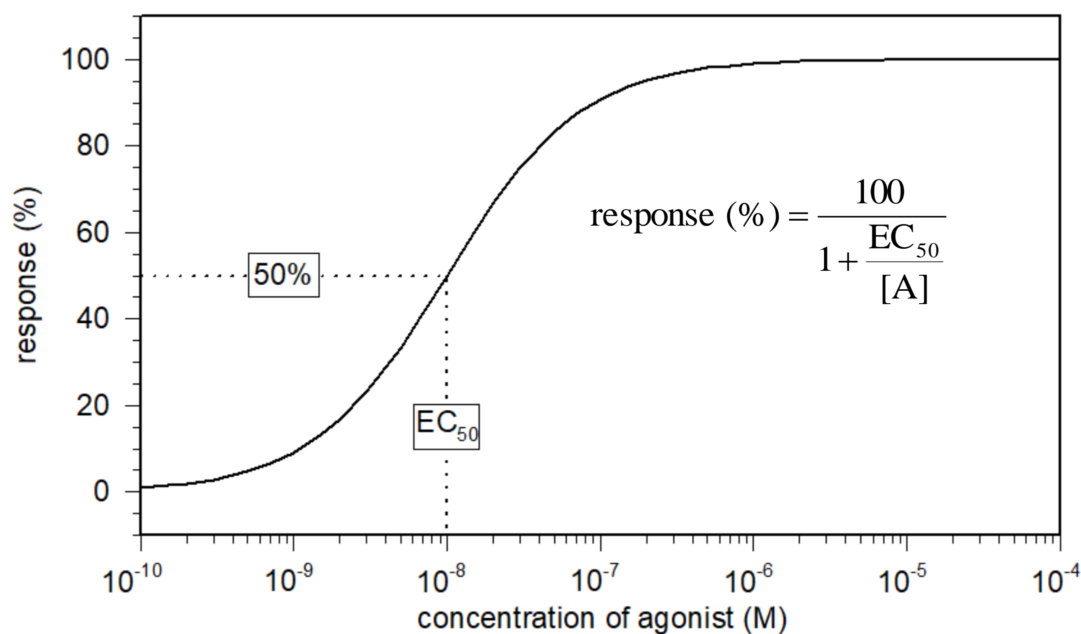

Fig. 3.1.: Agonist induced effect as a function of the agonist concentration ([A]) "dose-response curve"

Assuming that the response evoked by the ligand is proportional to the occupancy of the receptors, and the maximal response is reached when all receptors are occupied, EC_{50} equals to K_d. This simple rule, however cannot describe all ligand-receptor interactions.

According to a more advanced theory of the interaction, the biological effect of the ligand depends on two independent factors: the affinity of the receptor for its ligand and the intrinsic activity of the ligand. The latter shows how effective the ligand is in inducing the biological response after binding to its receptor. Namely, 1./ the pharmacological effect is not necessarily proportional to the number of occupied receptors, 2./ to reach maximal response the agonist does not need to bind to all receptors and 3./ ligands evoking the same response may give rise to different occupancies. In this framework EC_{50} is not necessarily equal to K_d but will depend on factors other than the affinity of the receptor.

Analyzing dose-response curves can reveal if an antagonism is competitive or not. In the presence of a competitive antagonist, the maximal response is unchanged, whereas EC_{50} and the K_d change (Fig. **3.3.**). In the presence of a noncompetitive antagonist, the maximal response decreases, EC_{50} might change but the K_d remains the same (Fig. **3.4.**).

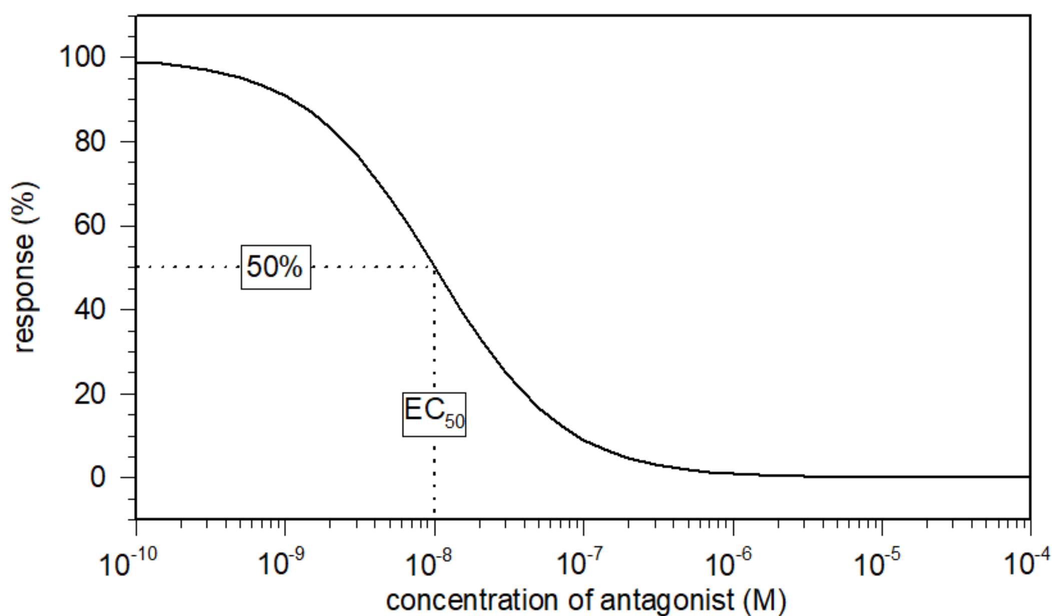

Fig. 3.2.: The response that can be evoked by different concentrations of an antagonist in the presence of a given concentration of the agonist

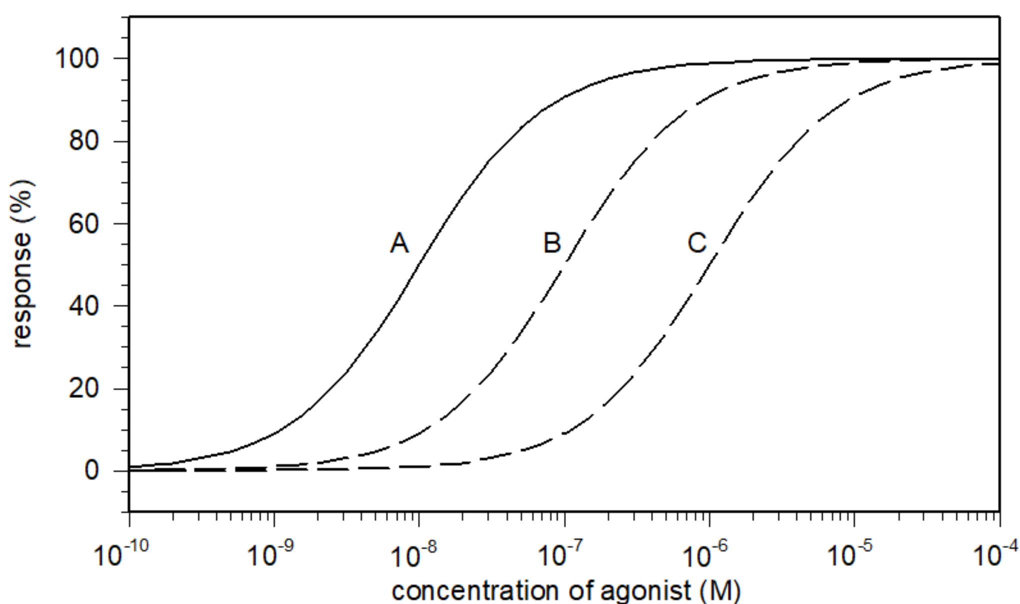

Fig. 3.3.: The effect of a competitive antagonist on the response evoked by different concentrations of the agonist

A: dose-response curve in the absence of the antagonist, B and C: dose-response curves in the presence of the antagonist. The concentration of the antagonist was greater in case of C than in B. Ordinate: the response was expressed as a percent of maximal response that can be induced by the agonist

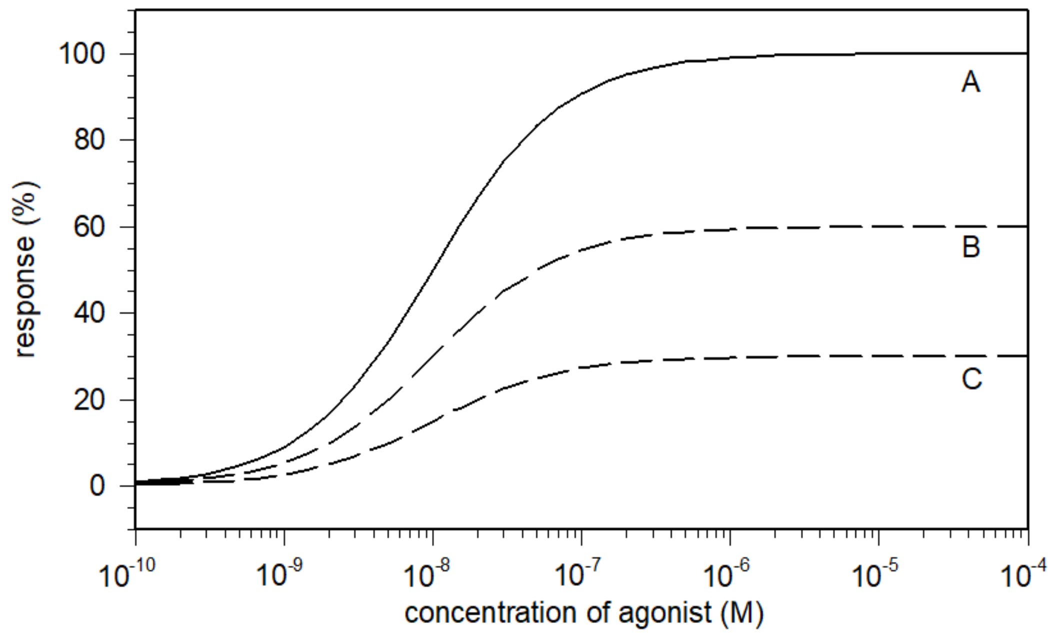

Fig. 3.4.: The effect of a non-competitive antagonist on the response evoked by different concentrations of the agonist

A: dose-response curve in the absence of the antagonist, B and C: dose-response curves in the presence of the antagonist. The concentration of the antagonist was greater in case of C than in B. Ordinate: the response was expressed as a percent of maximal response that can be induced by the agonist

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3.1. Simulation of the action potential and ionic currents in nerve fibers

3.1.1. Theoretical background

The staff of Axon Instruments, Inc. (USA) developed the program in 1989 based on the classical equations of Hodgkin and Huxley (1952). Though the original measurements were made on squid giant axon using voltage-clamp, the present simulation uses data, accumulated since, representing a mammalian nerve fiber.

The total transmembrane ionic current (I_i) on nerve fibers can be separated into three major components: Na^+ current (I_{Na}), K^+ current (I_K) and the current carried by all other ions (I_L). (On other excitable cells, other ionic currents may also contribute significantly to the total current, see chapter 3.2.)

$$I_i = I_{Na} + I_K + I_L \qquad (3.1.1.)$$

The actual size and direction of a given ionic current is determined by the **electrochemical gradient** as well as by the **conductance (G)** of the membrane for the given ion. Since the ionic concentrations usually do not change significantly during physiological excitatory processes, the change in the electrochemical gradient is due to the change in the membrane potential. The net driving force that determines the direction of the ionic flow is the difference between the actual membrane potential (V_m) and the equilibrium potential of the given ion (E_{ion}). The conductance of a unit surface on the membrane depends on the number of ion channels, on the unitary conductance of the channels and, finally, on the ratio of the open to the closed channels.

On the basis of the above, the ionic current carried by any given ion (I_X) can be calculated from Ohm's law as:

$$I_X = G_X * (V_m - E_X) \qquad (3.1.2.)$$

where: E_X - is the equilibrium potential for ion X,
G_X - is the specific conductance for ion X,
V_m - is the actual membrane potential.

According to the Hodgkin-Huxley model, the Na^+ and K^+ channels in the membranes of excitable cells are gated in a voltage and time dependent manner. Those ions which do not participate in the excitatory processes can be characterized by a constant conductance (leak, G_L).

3.1.2. The structure of the program

The program offers three different aspects (see below) of studying the electrical properties of excitable membranes. The selection between the possibilities

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can be made in the Main Menu, which is self-explanatory. Data can be entered or altered at any point of the simulation by choosing the data-entry menu, which explains the parameters used as well as the means of changing a given parameter.'

3.1.2.1. The voltage-clamp

The ionic currents (apart from the previously mentioned 'leak' current) show both time- and voltage-dependence, therefore, their exact behavior is hardly resolvable from measurements using action potentials (in this case the membrane potential is changing continuously). A so called **Voltage-clamp** circuit was introduced to overcome this problem which 'clamps' the voltage to a certain, preset value and, furthermore, enables to switch from one fixed membrane potential to another very rapidly. Following these rapid changes, the time dependence of a given current (or several currents at the same time) can be studied at the new, constant membrane potential.

3.1.2.2. The current-clamp

During current-clamp a constant current (the amplitude and duration are set by the user), is injected into cell and the changes in membrane potential displayed. This part of the simulation is for studying action potentials.

3.1.3. Aspects of the evaluation

3.1.3.1. The voltage-clamp

Kinetic analysis

Applying an appropriate depolarizing stimulus, there is a rapid increase in Na^+ conductance, which reaches a peak and then returns to its resting level. The maintained depolarization keeps the Na^+ channels in the inactivated state. In contrast, the K^+ conductance increases gradually and after reaching the maximum it remains constant; showing no inactivation.

Current-voltage relation

Upon increasing the depolarization, the peak of the Na^+ current first increases (becomes greater in absolute value), then at a certain membrane potential, even though G_{Na} is strongly activated, Na^+ current cannot be detected, because the equilibrium potential for Na^+ (E_{Na}, see Eqn. 3.1.3.) has been reached. Increasing the depolarization beyond the equilibrium potential will result in current flowing outward in contrast to the inward current seen with smaller pulses (the membrane potential where the current changes its sign is also called reversal potential). The K^+ current, on the other hand, will increase even when G_K is completely activated due to the fact that E_K is negative compared to the resting membrane potential thus the driving force for K^+ increases proportionally with increasing depolarization.

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3.1.3.2. The current-clamp

Determining the threshold, evoking an action potential

If the intensity of the stimulus does not reach the threshold, then the result is a local, or **electrotonic** response. (The actual waveform of this simulated electrotonic potential change differs somewhat from the physiological, since the program does not take all the passive properties of the membrane into account.) Stimuli exceeding the threshold evoke propagating action potentials with latency periods strongly dependent on the applied pulse amplitude. The activation of the G_{Na} plays the key role in the formation of the ascending phase of the action potential, while the inactivation of G_{Na} and the activation of G_K are responsible for the repolarization. After returning to the resting membrane potential, the K^+ conductance remains elevated for a while, and thus, contributes to the development of after-hyperpolarization.

Temporal summation

The electrotonic potential changes elicited by subthreshold stimulation do not have refractory periods, therefore subthreshold stimuli with appropriate timing will sum and an action potential will be generated.

Refractory periods

During the course of an action potential, there are phasic changes in the excitability of the membrane. During the ascending part and at the beginning of the repolarization, new action potentials cannot be evoked (maximally activated and partially inactivated G_{Na}). This phase is, therefore, called the absolute refractory period. The relative refractory period means that only stimuli stronger than the threshold are able to evoke a new action potential. This is explained by the fact that the K^+ conductance is still increased, relative to the resting state. In this case, the outflow of K^+ is opposing any tendency for depolarization, resulting in slower and smaller changes of the G_{Na}, as well as action potentials with shallower rising phases and smaller amplitudes. The excitability in the subnormal period is also smaller, simply because of the hyperpolarization.

The differences found emphasize the fact that the 'all-or-nothing' law for the amplitude of the action potential is valid only for the resting membrane, since action potentials from relative refractory periods show a wide variety in shape.

Repetitive action potentials

Information conveyed along nerve fibers is frequency coded. This is due to the fact that **long lasting stimuli** result in repetitive firing of the nerve, and that the frequency is the function of the amplitude of the pulse. The differences in the shape of the action potentials, when compared to the first one, can be understood on the basis mentioned in the section on the refractory periods. Repetitive activity can, however, be evoked not only by constant external stimuli but also by **decreasing G_K** (e.g. using TEA). This observation clearly demonstrates the important role of the K^+

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conductance in stabilizing the resting potential and illustrates that an external excitatory stimulus does not necessarily act by increasing the Na^+ conductance.

The strength-duration curve

Only those stimuli will evoke action potentials that develop with enough speed (see accommodation) and reach a required intensity. Short stimuli will not excite the membrane independently of their amplitude, while stimuli with small amplitude will not evoke action potential even if their duration is long.

The term **rheobase** was introduced to mean the smallest amplitude that is capable of activating the membrane if its duration is long enough (infinite). For pulses greater than the rheobase, increased stimulus amplitude necessitates less time to evoke a regenerative response. (The time corresponding to the pulse with amplitude twice the rheobase is called **chronaxia**.) The relationship between the amplitude and the duration of the stimulus is called 'strength-duration curve', which in the present situation, is a current strength-duration curve.

Ion substitution

Increasing or decreasing the extra- or intracellular concentration of a given ion, according to the Nernst equation, results in **equilibrium potential changes**. Since only the ratio of the above concentrations is what counts, the program allows this ratio to be changed relative to the physiological situation. This will, naturally, alter the driving force of the given ion (Eqn. 3.1.3.).

Changing the concentration of Na^+ will thus affect the rate of rise and the maximal amplitude of the action potentials. The resting membrane potential, due to the low resting G_{Na}, is hardly influenced. The relatively greater G_K causes the resting membrane potential to be extremely sensitive to changes of the external K^+ concentration; resulting in repetitive firing, long lasting depolarization or hyperpolarization. The direct effect of the K^+ concentration changes on the action potential mainly alters the repolarization phase. Nevertheless, by changing the resting membrane potential and, consequently, the resting inactivation of the Na^+ channels, the rising phase and the amplitude of the action potentials will also be altered.

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3.2. Computer simulation of cardiac action potentials

3.2.1. Theoretical background and the model

The program is based on the model of Beeler and Reuter (1977) developed for ventricular cardiac muscle cells. Their description of the cardiac action potential is an adaptation of the model of Hodgkin and Huxley. The theoretical basis of the simulation thus agrees in its basic assumptions with that described in chapter **3.1.1.**, and therefore, only the differences will be pointed out rather than reviewing the entire material.

3.2.1.1. Fast action potentials

The so called fast action potentials can be recorded from some of the elements of the cardiac conducting system (e.g. Purkinje fibers) and from the contracting cells of the heart. The fast action potential, according to the Beeler and Reuter model, is the result of the opening and closing of four separate ionic channels.

The **ascending part** is due to activation of the **fast sodium channels.** The voltage- and time-dependence of the Na^+ conductance can be calculated using Eqn. 3.1.5.

In the formation of the beginning of the **plateau phase**, a slow inward current, (I_S) primarily carried by Ca^{2+}, is thought to play a fundamental role. These channels are, however, also permeable for Na^+, but to a lesser degree. Their activation can be prevented by the application of Mn^{2+}, verapamil or other calcium channel blockers.

The conductance of the slow inward current (G_S) can be calculated in the following way:

$$G_S = G_{S,max} * d * f \quad (3.2.1.)$$

where: $G_{S,max}$ - the maximal conductance of the slow channels,

d, f - dimensionless parameters with values between 0 and 1 (d for the activated and f for the non-inactivated channels).

The slow ionic current influences the transmembrane Ca^{2+} concentration gradient (due to the low intracellular Ca^{2+} concentration and the relatively long lasting Ca^{2+} entry), thus E_{Ca} varies with time. These changes were incorporated into the model. (The resting intracellular Ca^{2+} concentration was taken to be 0.3 mmol/L.)

In the formation of the later plateau phase (i.e., in the maintenance of depolarization), the **decrease of the resting K^+ conductance** might also play a role together with the inactivation of the slow calcium current. These K^+ channels close upon depolarization decreasing the repolarization tendency caused by the outflow of K^+.

The most important factor in **repolarization** is the activation of the **voltage- and time-dependent K^+ conductance**. Depolarization opens these K^+ channels resulting in repolarization due to the efflux of K^+. Moreover, repolarization is assisted by the inactivation of the Ca^{2+} conductance as well as by the return of the resting K^+ conductance to its original level.

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3.2.1.2. Slow action potentials

The so-called **slow action potentials** can be recorded from the cells of the **sinoatrial** (SA) and the **atrioventricular** (AV) **nodes**. Slow response can also be evoked from cardiac cells that (under physiological conditions) generate fast action potentials, if the fast Na^+ conductance is eliminated (e.g. by inactivating the Na^+ conductance with prolonged depolarization or by tetrodotoxin-treatment). The ascending phase of a slow action potential is due to the opening of the slow channels (carrying Ca^{2+} and Na^+). Ca^{2+} entry is an important factor in the maintenance of the prolonged depolarization (plateau), just as the decrease in the resting K^+ conductance. The activation of the voltage- and time-dependent K^+ conductance is the fundamental factor in producing repolarization, together with the inactivation of the Ca^{2+} conductance, as well as the return of the resting K^+ conductance to its original level.

Neither the cells of the SA nor the AV node maintain a stable resting membrane potential. Upon reaching a maximal repolarization value (about -60 mV), a spontaneous slow depolarization (prepotential) begins which, after reaching a threshold value, results in an action potential. The mechanisms underlying the development of the prepotential vary depending on the species and cell type. The activation of ionic channels with slow gating and carrying inward currents (I_{Ca}, I_{Na}), or the decrease of K^+ conductance will equally result in depolarization. In the simulation program, the introduction of a steady Na^+ current causes the spontaneous, repetitive activity. The appearing action potentials can be considered as slow response, since the maximum diastolic potential of the simulated cell is -50 mV (i.e., the fast Na^+ conductance is inactivated).

3.2.2. Aspects of the evaluation

Determination of stimulus threshold

When determining the threshold, follow the description given in chapter 3.1.3., bearing in mind that in this case it is not an inward current that causes depolarization but rather a sudden change in the membrane voltage at time zero. When studying the events during the action potentials, note that only the 10 ms simulation period makes it possible to study the ascending phase of the action potential, i.e. the activation of the fast Na^+ conductance (G_{Na}), and the decrease in the resting K^+ conductance. Increasing the simulation period to 350 ms, while keeping the other parameters constant, the conductance changes and the accompanying ionic currents during the plateau and the repolarization can be studied (at this time scale, the fast Na^+ conductance and the Na^+ current cannot be seen).

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Depolarization block

The heart operates as a functional syncytium and thus the electrical status of a cell will influence that of its neighbors. This is the case even when a cell has a sustained membrane potential of 0 mV (due to, for example, a sustained oxygen deficit) and decreases the resting potential of the adjacent cells. As this effect acts as a continuous depolarization, this will inactivate most of the fast Na^+ channels and prevent the development of the propagative action potential (depolarization block).

Effects of changes in the resting potential

Choosing a resting potential between the normal and the one that evokes depolarization block, action potentials with different kinetics (slower rising phase and smaller amplitude) can be generated. The underlying mechanism is the partial inactivation of the fast Na^+ channels due to the prolonged depolarization.

Effects of adrenergic and cholinergic stimulation on the atrial and ventricular myocardium

Adrenergic stimulation of the **myocardial cells** of the atria and ventricles, increases the excitability and conduction velocity (positive bathmo- and dromotropic effects, respectively), and results in a positive inotropic effect (i.e., an increase in the contractile force). The positive inotropic effect of catecholamines is explained by an increase in the conductance of the voltage gated Ca^{2+} channels (cAMP mediated channel phosphorylation is the likely reason), allowing more Ca^{2+} to enter the intracellular space during the action potential. As a consequence, the intracellular Ca^{2+} concentration is increased, resulting in a greater activation of the contractile system. The program simulates the positive inotropic effect by increasing the intracellular Ca^{2+} concentration. In contrary, the effect of cholinergic stimulation is modeled by decreasing the intracellular Ca^{2+} concentration.

The effect of adrenergic and cholinergic stimulation on the pacemaker cells

Increased activity of the sympathetic nervous system has positive bathmo-, chrono- and dromotropic effects on the pacemaker and conducting elements (**nodal tissue**) of the heart. This is explained by a shift of the maximal diastolic potential towards the critical potential for action potential generation, resulting in an increase in the rate of rise of the prepotential. Upon cholinergic stimulation, the above parameters change in the opposite direction, and negative tropic effects are observed.

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3.3. Computer simulation of Frank-Starling-mechanism

3.3.1. Theoretical background

The heart is a pump whose performance can be characterized by the amount (volume) of blood pumped in one minute (minute volume or cardiac output). This volume is equal to the volume pumped from the left ventricle into the aorta in one minute. The right ventricle pumps the same amount of blood into the arteria pulmonaris as the left ventricle into the aorta. The cardiac output (mL/min) is equal to the product of the stroke volume (mL) and the heart rate (bpm).

The actual value of the cardiac output depends on several factors like body surface area, emotional state, physical work etc.

As stated above, the cardiac output is determined by two factors: heart rate and stroke volume. If both factors change in the same direction (either increase or decrease) the cardiac output will change in that direction, too. If this is not the case, then the stronger change will determine the direction of change in the cardiac output. One possibility of regulation is related to an **intrinsic** property of cardiac muscle. The cellular basis of this regulation is the length-tension diagram (Fig. **3.5.**). This intrinsic aspect can be best studied using an isolated heart-lung preparation called the Frank-Starling-preparation in which the autonomic innervation of the heart is missing. A more detailed description may be found in the textbook.

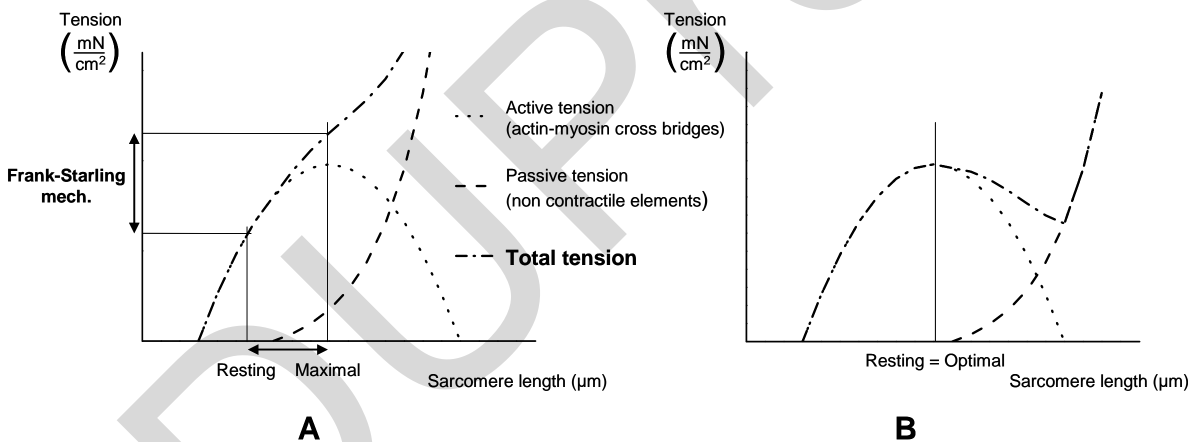

Fig. 3.5.
The length-tension diagram of the cardiac (A) and skeletal muscle (B).

In skeletal muscle the resting sarcomere length is equal to the optimal sarcomer length (therefore, the increase in sarcomere length does not lead to increase in tension), while in cardiac muscle the lengthening of the sarcomere leads to the increase of tension (due to the increased number of cross-bridges and the sensitisation of myofilaments to calcium).

In case of load (physical work, emotional load, insufficient oxygen tension, etc.) the escalated metabolism of the body tissue can be maintained only at a higher cardiac output. The Frank-Starling-mechanism is **one of the possible ways** to achieve this goal; the heart is capable of increasing its stroke volume, therefore, the

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cardiac output will also increase at a constant frequency. This mechanism is evoked by the increase of the venous return (venous load), and because the heart utilizes its **diastolic reserve**. In a resting person the ventricular end diastolic volume is about 140 mL, while the end systolic volume is about 60 mL, so the stroke volume is 80 mL. The venous return can be increased up to 260-300 mL by the increase of venous pressure. The end diastolic volume will increase in a lesser extent so the stroke volume will also increase. In the case of increasing the peripheral resistance the compensation happens similarly. The end-diastolic volume and the end-systolic volume increase in the same degree, resulting in an unchanged stroke volume. The isolated or denervated heart has only this mechanism to adjust its performance. Using this mechanism a human cardiac output can be roughly doubled to 10 L/min.

In case of elevated metabolic demands, the blood vessels dilate, their resistance decreases and the blood flow increases. The main regulating factor is the venous return. As long as the venous return increases less than twice of the resting value, the Frank-Starling-mechanism is capable of increasing the end-diastolic and end-systolic pressures in the same way, and this results in an increase of the stroke volume.

One of the best parameters to monitor the effectivity of cardiac pumping is the ejection fraction (EF) which can be calculated as the ratio of the stroke volume and the end diastolic volume (EF= SV/EDV). Normally its value is between 0.55-0.60, while it can be lower than 0.4 in heart failure.

Shock is the failure of peripherial circulation as the blood volume and the capacity of blood vessels becomes disproportional to each other. Its leading symptom is the progressive reduction of blood pressure which leads to death without proper intervention. The arterial blood pressure mainly depends on the total peripheral resistance and the cardiac output. One of the most sensitive indicator of developing shock is the shock-index which can be calculated as the ratio of heart rate and systolic pressure (SI=HR/Ps). Normally its value is between 0.5 and 0.7. Increasing above 1 means greater risk for developing and progressing shock.

3.3.2. The program simulates the Frank-Starling-mechanism at constant frequency calculating the typical parameters of the functioning human heart. Modifying (within reasonable limits) two parameters such as the venous return and peripheral resistance can influence the performance of this simulated heart. Based on the given values of these data, and taking into account the initial parameters of the circulation, the program calculates, from beat to beat, the temporal changes of the cardiac parameters, such as tension of the ventricular wall, ventricular outflow, ventricular pressure, and ventricular volume. One can set two functions of the calculated ones, which can be presented in the screen at any given time showing the time dependence of the chosen function in dynamic mode.

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3.3.3. Aspects of evaluation

3.3.3.1. Effect of preload

Elevation of the venous reservoir of the Frank-Starling-preparation increases the venous (loading) pressure and results in a higher volume loaded into the right ventricle. During diastole the end-diastolic volume increases. Due to the large capacity of adaptation to variation of venous return, the heart is able to pump the required amount of blood from the right (venous) side to the left (arterial) side of the heart at a *constant frequency.* For this reason, the cardiac output as well as the pulse volume (stroke volume) will change according to the preload.

Following a sudden increase of venous (loading) pressure, an additional extra volume appears in the heart. It requires a few (3-5) additional strokes to get the balance again between the venous return and the stroke volume. Increasing the venous pressure will result in an increase of the end-diastolic volume and ultimately in the end-systolic volume. After a few strokes, a new balance will be re-established between the venous load and the stroke volume at a higher volume. Since the program simulates a constant beating frequency, the higher cardiac output is a direct result of the higher stroke volume.

3.3.3.2. Effect of change in the peripheral resistance

If the peripheral resistance decreases below a certain value, the coronary flow will also decrease. When falling below a certain limit, the coronary flow becomes insufficient, and the pumping function of the heart is substantially damaged due to the lack of proper blood perfusion (myocardial infarction). On increasing the peripheral resistance, without changing the venous return, the heart will forward the amount of blood from the venous (right) side into the arterial (left) side of the circulation. Under steady state conditions the cardiac output remains constant even if the peripheral resistance has been changed. However, the establishment of the new equilibrium after a sudden change of the peripheral resistance requires a few cardiac cycles. In the early phase of this adaptation the venous return is higher than the stroke volume causing a gradual increase of the ventricular volume. The end-systolic and the end-diastolic volumes increase until the required balance is achieved again, when the pulse volume reaches the value identical before the sudden change of the peripheral resistance.

3.3.3.3. Circulatory shock

Using the program, you can demonstrate the main features of the peripheral circulatory insufficiency called shock. On decreasing the peripheral resistance, you can evoke low arterial blood pressure, which causes progressive processes leading to death.

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3.4. Simulation of renal transport mechanisms

3.4.1. Theoretical background

This program simulates and demonstrates the passive and active processes involved in the formation of urine, using characteristic substances.

3.4.1.1. Inulin-like passive transport

Inulin, a plant polysaccharide, is freely filtered into the glomerular ultrafiltrate and is not affected by tubular transport mechanisms, like tubular reabsorption or secretion. As a consequence of free filtration, the concentration of a given substance in the ultrafiltrate equals to the concentration of the same substance in the plasma. The amount that is filtered in a one-minute interval can be calculated from the glomerular filtration rate (GFR) and from the plasma concentration (P) of the given substance:

$$\text{filtered amount} = GFR * P \qquad (3.3.1.)$$

The excreted amount can be given if we multiply the diuresis (V) by the final urinary concentration (U):

$$\text{excreted amount} = U * V \qquad (3.3.2.)$$

Since inulin-like substances are neither secreted nor reabsorbed, the filtered and excreted amounts are the same:

$$GFR * P = U * V \qquad (3.3.3.)$$

Rearranging gives (Based on: $C=U*V/P$):

$$GFR = C \qquad (3.3.4.)$$

where C is the clearance of inulin.

3.4.1.2. Active transport mechanisms

The tubular epithelium is able to transport certain materials from the peritubular capillaries into the tubular fluid (**PAH-like active secretion)**, and can subtract others from the tubular fluid (**glucose-like active reabsorption**). These changes can substantially alter the final composition of the urine, compared to the ultrafiltrate.

Para-amino-hippuric acid (PAH) is filtered in the glomeruli and secreted in the tubules. The characteristics of the ultrafiltration of PAH are the same as those of inulin. The amount of PAH secreted can be determined indirectly by subtracting the filtered amount from the excreted one:

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$$\text{secreted PAH} = \text{excreted PAH} - \text{filtered PAH}, \qquad (3.3.5.)$$

or using the notations from Eqn. 3.3.1. and 3.3.2.:

$$\text{secreted PAH} = U_{PAH} * V - GFR * P_{PAH}. \qquad (3.3.6.)$$

Naturally, the excreted amount is always greater than that of the filtered.
Furthermore the PAH clearance can be calculated from the following equation: $U_{PAH} * V = P_{PAH} * GFR + \text{secreted PAH}$ if the whole equation is divided by P_{PAH} resulting in:

$$C_{PAH} = GFR + \text{secreted PAH} / P_{PAH} \qquad (3.3.7.)$$

Glucose-like substances (e.g. glucose and other monosaccharides, amino acids) are freely filtered through the glomerular membrane, but do not appear in the urine under physiological conditions, because they are actively reabsorbed from the tubules. The reabsorption is a carrier-mediated mechanism and, therefore, it can be saturated (Tm_G).

The amount reabsorbed can be calculated from the filtered and excreted amounts:

$$\text{reabsorbed glucose} = GFR * P_G - U_G * V, \qquad (3.3.8.)$$

that is, at low plasma glucose concentrations (P_G), when the excreted amount is zero, the reabsorbed glucose will be proportional to P_G.

To determine the transport maximum (Tm_G) of any given substance, the plasma level should be high enough to saturate the transport system. In this case, the maximal amount transported can be calculated using equations 3.3.6. or 3.3.8. GFR is measured as the inulin or endogenous creatinine clearance.
Glucose clearance can be calculated from the following equation:
$U_G * V = P_G * GFR - \text{reabsorbed glucose}$ if the whole equation is divided by P_G resulting in:

$$C_G = GFR - \text{reabsorbed glucose} / P_G \qquad (3.3.9.)$$

3.4.2. Aspects of evaluation

To determine the renal plasma flow (RPF) the following calculations have to be considered: RPF = RBF * (1-Htc), where RBF is the renal blood flow and Htc is the value of hematocrit measured as a ratio and not in percentage; on the other hand the determination of effective renal plasma flow (ERPF) is more important in evaluating renal function: ERPF = 0.9 * RPF
The value of ERPF can also be calculated from the GFR/FF quotient, where FF is the filtration fraction.

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3.4.2.1. Inulin-like passive transport

The reason that the **clearance** of the inulin-like substances can be used to determine the GFR, a characteristic parameter of glomerular function, is that it **is independent of the plasma level** since the ratio of the filtered and excreted amounts is constant. This law is simulated in the first part of the program, where one can examine the decay of inulin concentration in the plasma as the function of time at different initial plasma concentrations. We can follow the excreted amount (which is the same as the filtered amount) as the function of time, and the excreted amount of inulin as the function of P. It is shown that the excreted amount decreases proportionally with the decrease in P, so the clearance remains constant.

In clinical practice, after a single injection of a higher dose of inulin, a constant plasma concentration is maintained by continuous infusion, since the decrease in plasma level is not only due to filtration, but also to an equilibration with the extravasal fluid.

In certain diseases, when either the filtering surface of glomeruli or the renal blood flow decreases, the reduced GFR results in a decreased inulin excretion.

3.4.2.2. PAH-like secretion

It follows from equation 3.3.6. that the serum concentration of PAH rapidly decreases after the application of a single dose due to filtration and secretion. The curve representing the time-dependence of the **filtered amount** is identical to the curve characterizing the changes of the **serum level**, showing a **linear relationship** of the two values.

If the secretion transporters are not saturated, PAH is almost completely extracted from the plasma through filtration and secretion and, therefore, the extraction coefficient (E) is close to 1. Due to local differences in kidney perfusion, some glomeruli are left out from the excretion process, thus a value of 0.9 can be assumed for the extraction coefficient, when **determining the renal plasma flow (RPF)** using the PAH clearance:

$$RPF = C_{PAH} / E_{PAH} \qquad (3.3.8.)$$

RPF could be calculated from the clearance and the extraction coefficient of any substance, but the determination of the extraction coefficient is impossible in clinical practice. The reason for using PAH for the determination of RPF is that its extraction coefficient may be assumed to be 0.9 and only its clearance is to be measured. For this assumption to be valid, the plasma concentration should be such that the maximum capacity of tubular transport is not exceeded during the investigation. If there is a possibility of tubular damage, first Tm_{PAH} should be determined (using the method described in chapter 3.3.1.2.), then a definitely lower than saturating concentration of PAH should be administered for the determination of RPF.

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3.4.2.3. Glucose-like reabsorption

Notice the fact that the concentration dependence of glucose reabsorption shows saturation. At low plasma glucose levels, the rate of reabsorption is proportional to the concentration of the plasma (chapter 3.3.1.2.), then it becomes constant. The **splay** before reaching TmG is mainly due to the different transport maxima of the individual nephrons.

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3.5. Computer simulation of the glucose tolerance test

3.5.1. Theoretical background

Simulation of the interaction between plasma glucose and insulin following the infusion of test doses of glucose provides an insight into the physiological mechanisms of glucose metabolism. The **glucose tolerance test** is used to evaluate the ability of the pancreas to release insulin in response to a large dose of glucose given either orally or intravenously. In the clinical practice a blood sample is taken to determine the fasting blood glucose level, then the patient have to take 75 g glucose *per os* and two hours later another sample is taken to measure blood glucose level again.

The healthy pancreas releases enough insulin to lower the plasma glucose within a few hours, sometimes to the point of producing hypoglycemia. The **posthyperglycemic hypoglycemia** illustrates the transient properties of the homeostatic adjustments operating with negative feedback, i.e., the presence of damped oscillations around the physiological values.

The glucose tolerance test is very useful in diagnosing diabetes mellitus and other, milder pathological conditions in carbohydrate metabolism. The insufficient release of insulin or the increased secretion of hormones having insulin-antagonistic effects (glucocorticoids, thyroxin, epinephrine, growth hormone, etc.) causes a **decreased glucose tolerance** in diabetes mellitus and in diseases such as hyperthyroidism, Cushing's syndrome, growth hormone overproduction etc.

The state when the situation is just the opposite is called **increased glucose tolerance**. In this case, the initial glucose concentration is lower than the normal value and after glucose administration its rise is smaller and its fall is faster than in the physiological case. This can be caused by various diseases, e.g. an insulin-producing tumor in the pancreas (insulinoma), hypothyroidism, Addison's disease, hypopituitarism, etc.

A more sensitive version of the glucose tolerance test is the **Staub-Traugott method** involving a double glucose administration: the first glucose load is followed by a second one in the 90th minute. Using this method, differences between the normal and pathological curves will become more pronounced.

The mathematical model of the simulation program is identical to the one presented by Stolwijk and Hardy (1974).

The model includes two inputs of glucose:

1. The glucose, released by the liver due to the glycogenolysis, is assumed to be constant (8400 mg/h in the normal state). This data is referred to as the **liver constant** in the following description.

2. The infusion of exogenous glucose for the tolerance test. It is assumed to be constant in the program (80000 mg/h). The infusion lasts for 30 min, thus provides 40 g glucose in the single load version. Using the Staub-Traugott method, the total glucose intake is 80 g.

Three mechanisms are considered to lower the glucose concentration:

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1. Loss due to the excretion by the kidney, when the plasma glucose concentration exceeds the transport maximum of the tubular transport system (the renal threshold is 13.88 mmol/L in the case of normal transport capacity).

2. The plasma glucose concentration is decreased by the insulin-insensitive glucose uptake of the cells (depending only on the plasma glucose level).

3. Insulin-sensitive glucose uptake by the skeletal and smooth muscle, as well as adipose tissues. It depends both on the plasma glucose concentration and on the insulin activity of the blood.

The reactivity of the pancreatic islet cells to the increased plasma glucose concentration is characterized by the pancreas constant (the normal reactivity is 100% in the program). The pancreas constant indicates the rate of increasing of insulin activity as a response to the elevated blood glucose concentration. Insulin is eliminated from the blood through degradation by the insulinase enzyme.

3.5.2. Aspects of evaluation

3.5.2.1. Normal tolerance

Single load: the blood glucose concentration and the insulin activity is in the physiological range; the glucose concentration returns to its initial value within 2 hours after the load, and then a mild hypoglycemia develops.

Double load: the second glucose peak will be smaller than the first one due to the already elevated insulin activity (initiated by the first load), and the insulin activity further increases is due to the second glucose administration leading to larger glucose level lowering effect.

3.5.2.2. Decreased glucose tolerance

Single load: the initial glucose level of the blood is elevated compared to the physiological value and, in spite of this, the insulin activity is lower than in control. After glucose administration, the time necessary to reach the initial value is more than 2 hours, and the posthyperglycemic hypoglycemia is smaller or totally absent.

Double load: the second glucose peak will be higher than the first one, since the elevation in insulin activity is much less pronounced.

3.5.2.3. Increased glucose tolerance

Single load: the initial blood glucose concentration is low, while the insulin activity is high. The blood glucose concentration rapidly returns to and falls below its initial value. The posthyperglycemic hypoglycemia can reach critical values causing severe problems.

Double load: the second peak in insulin activity substantially exceeds, while the second peak in the blood glucose concentration is much smaller than the first ones.

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3.5.2.4. Renal threshold

Reduced: a significant amount of glucose is lost in the urine. In acute conditions, this alteration does not affect the normal characteristics of the glucose tolerance (e.g. phloridzin-treatment in a short-term animal experiment), but a long-lasting reduction in the renal threshold may cause a significant glucose loss, resulting in a lowered insulin activity as a compensatory mechanism.

Elevated: has no significant effect on the glucose level.

3.5.2.5. Liver constant

Reduced: after depriving the system from its major source of glucose, a serious reduction of the blood glucose concentration will develop, therefore, the posthyperglycemic hypoglycemia will be more pronounced.

Elevated: can lead to hyperglycemia accompanied by a higher insulin activity.

3.5.2.6. Pancreas constant

Reduced: states of decreased glucose tolerance of different degrees can be simulated.

Elevated: will result in states of increased glucose tolerance of different degrees.

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3.6. Investigation of processes in cell physiology using computer simulation

3.6.1. Theoretical background

Computer programs in this group simulate the effects of various agents on isolated preparations. The biological effects of the agonists and antagonists acting on the receptors expressed in the given tissues will be investigated. Furthermore, the program allows the identification of "unknown'' compounds, resembling either the agonists or antagonists. This part of the project simulates the situation that is often faced in real research, when the biological responses evoked by new substances are tested.

3.6.1.1. Investigation of the humoral regulation of intestinal smooth muscle

This program simulates the effects of various drugs on an ileum loop isolated from a guinea pig. Since this type of smooth muscle shows no or very little spontaneous activity, the effects of the applied agonists and antagonists can be easily monitored by recording the mechanical activity of the tissue.

In the first set of experiments, we shall investigate the effects of acetylcholine on the contractile properties of the simulated ileum loop. The effect is mediated via the third subclass of the muscarinic-type cholinergic receptor (M_3). The binding of acetylcholine to its receptor causes - through the activation of the IP_3 second messenger pathway - an increase of the intracellular calcium concentration, resulting in the contraction of the intestinal smooth muscle. Atropine can antagonize the effect of acetylcholine on muscarinic receptors, as it is capable of binding to the same receptor, without activating it.

The experiments will investigate the effect of another well-known antagonist of the cholinergic receptors, called hexamethonium. Hexamethonium exerts its effects on a completely different class of receptors, as it inhibits the neuronal type of the nicotinic acetylcholine receptor. These are found on the cell bodies of postganglionic autonomic neurons regardless of the fact whether they belong to the sympathetic or parasympathetic nervous system. The same receptors are found on the chromaffine cells of the adrenal medulla. The only similarity between the nicotinic and muscarinic type acetylcholine receptors is that both are stimulated by acetylcholine, but neither the mechanism of the action nor the pharmacology is the same in these two classes of receptors.

The function of the synapses where neurotransmission involves the release and action of acetylcholine can be affected by drugs that inhibit the enzyme responsible for breaking down acetylcholine. This enzyme is called acetylcholine-esterase, and it is one of the fastest working enzymes in the human body. The main function of this enzyme is to swifty remove acetylcholine from the synaptic cleft and thereby stop uncontrolled synaptic activity. Taking into account the significance of this enzyme, it is not entirely surprising that drugs causing irreversible inhibition of acetylcholine-esterase are the usual constituents of pesticides and substances used in chemical

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warfare. In the present experiments, the effects of physostigmine will be investigated. It causes a reversible inhibition of the acetylcholine-esterase, hence it has a much milder effect which can be of importance in the clinical practice.

Numerous other drugs are used in the clinical practice which affect smooth muscle function, one of these is the well-known smooth muscle relaxant papaverine. It is believed that papaverine causes a rather effective relaxation of the smooth muscle by inhibiting the phosphodiesterase and the entry of Ca^{2+} into the smooth muscle cells.

It is of practical significance that histamine released from mast cells is a powerful stimulator of the intestinal smooth muscle. The activation of the H_1 class histamine receptors is also linked to the IP_3 signaling pathway, explaining the strong increase of the intestinal smooth muscle motility demonstrated in this program.

As we shall see, not only the applications of various drugs, but also a simple maneuver such as altering the extracellular K^+ concentration can also greatly influence the activity of the investigated smooth muscle by changing its resting membrane potential.

3.6.1.2. The role of endothelial cells

This program enables to study the effects of different agents on isolated vascular rings. The response of arterial rings with or without intact endothelium and of venous rings with intact endothelium can be elucidated.

The effects of norepinephrine and acetylcholine as neurotransmitters will be investigated on vascular rings with or without endothelium. The mediator function of nitrogen monoxide will be characterized indirectly through the inhibition of nitric oxide synthase (NOS) (by using L-NMMA). To elucidate the non-adrenergic non-cholinergic neuronal vasodilator mechanisms, the effects of a possible neurotransmitter - substance P - will be analyzed.

3.6.1.3. Synaptic transmission in the neuromuscular junction

The neuromuscular junction (NMJ) is a specialized synapse between the motoneuron and the skeletal muscle in which the acetylcholine is released as a neurotransmitter. Through binding to cholinergic receptors in the end-plate membrane, the acetylcholine evokes an end-plate potential, which in turn initiates the subsequent steps of excitation-contraction coupling resulting in the shortening of the muscle fiber.

The program package offers several different preparations to simulate the basic characteristics of the NMJ. The first preparation is the rectus abdominis muscle of the frog, which is a multi-innervated tonic muscle. Agonists of the end-plate will thus induce depolarization and a subsequent contracture. In the program, the mechanical response (increase in tension) to various agonists is measured and the cholinergic receptor can be characterized. The other preparation is the phrenic nerve (diaphragm of the rat), which is often used in the *in vitro* laboratory measurements, too. In this experiment, the mechanical response can be evoked not only by adding

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cholinergic agonists, but also by stimulating the nerve. Isolated contractions are evoked by supramaximal stimuli of 0.2 ms in duration applied at a rate of 0.05 or 0.5 Hz. Tetanus can also be evoked (stimulation at 30 Hz for 5 s).

3.6.1.4. Identification of an "unknown" pharmacon

One of the many advantages of the above programs is that they offer the possibility of investigation of the effects an "unknown" pharmacon and its identification. This simulates the common situation when the mechanisms of action of a new compound are to be determined.

Before starting the experiment, a clear question should be put forth. A question is good if an experimental protocol can be assigned to it, which will give a definite answer. Based on previous knowledge one should form working hypotheses, which can be either confirmed or discarded based on the data obtained.

The "unknown" substance will have an effect similar to one of the known agonists or antagonists. In case of positive identification, this can be the basis of formulating our opinion (e.g. the "unknown" compound has acetylcholine-like action).

Let us now see the steps of identification. First one should try to evoke a response using a relatively small (e.g. 1 μmol/L) concentration to begin with. If this was ineffective, raise the concentration until reaching the effective dosage. Having reached it, a dose-response curve should be constructed and the EC_{50} value should be determined. This will be used to describe the efficacy of the substance. We should now form a working hypothesis on what type of substance we are using and what receptor might be involved.

The next step is the identification of the receptor. Using known agonists or antagonists of the various receptors one should decide between possible alternatives. If none of the agonists or antagonists seems to work, we should modify the working hypothesis and consider an action through voltage gated channels and test, therefore, blockers of the channels.

If we have a definite idea of what we are dealing with, the parameters of the dose-response curve of the "unknown" ligand should be compared to that of a known analogous ligand. This will enable us to characterize the potency and efficacy of the substance.

3.6.2. The structure of the programs

After starting the appropriate simulation program, read the general instructions, choose the color display (1), and the screen as the output (S). Follow the instructions in the topic sheets when selecting the units of measure. After choosing the preparation (where this is necessary), the list of applicable agonists or antagonist is displayed. If too many interventions were applied or the length of the experiment became too long, the responsiveness of the preparation declines or sometimes completely disappears. In this case a new preparation should be used by selecting the "Discard the preparation" option. The experiment can be then continued on a new preparation (in this case the "unknown" compound offered by the program remains

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the same), or you can be exit the program. Note, that on re-starting the simulation the program will give a new “unknown” compound.

3.6.2.1. Investigation of the humoral regulation of intestinal smooth muscle

The agonists (i.e. acetylcholine or histamine) are added into the bathing solution and then they exert their effects. After each application, the agonist is automatically removed (washed out), the tissue relaxes to its resting state (indicated by a short baseline), and is then ready for the application of the next concentration of the appropriate agonist. If an antagonist (e.g. atropine) is added, it remains in the bathing solution until 'Washout' is selected, hence the effect of an agonist, added during this time, will be affected by the antagonist present.

3.6.2.2. The role of endothelial cells

After adding the agonists into the bathing solution, they will remain there until the “Washout” option is selected. The antagonists on the other hand, can only be removed from the solution with the “Replace Krebs” option. The preparation has no basal tension, therefore, the effects of a relaxing agent can only be studied after a contraction is initiated by the addition of an agonist.

3.6.2.3. Synaptic transmission in the neuromuscular junction

In the program ‘**rectus abdominis’**, the agonists, after exerting their effects, are automatically washed out. Antagonists, however, will only be removed from the bathing solution if the “Washout” option is selected. A short message displayed on the screen reminds the user if there is an antagonist present.

The program ‘**phrenic-diaphragm**’ has a preset 0.05 Hz stimulation. The resulting contractions of the muscle are displayed as dashed horizontal lines. The stimulation will continue until a key is pressed. At this point, the experiment can either be continued with the same conditions, or the user can enter the option menu. The menu that appears enables the user to change the parameters of the stimuli or to apply different agents. If a new compound is applied, the stimulation cannot be stopped (WAIT message) until the effect of the drug fully develops.

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3.7. Computer simulation of skeletal-muscle function

3.7.1. Theoretical background

Physiology of skeletal muscle function is demonstrated in this simulation and can be studied from the official book: A. Fonyó: Principles of Medical Physiology where a whole chapter describes the skeletal muscle function. Reading this is absolutely recommended before starting the practice. The aim of the experiments is to make the student understand:

A. *How does an electrical stimulus evoke contraction of the muscle?*
B. *Why is there a relationship between the stimulus intensity and contractile force?*
C. *What is the relationship between the initial length of the muscle fibers and the force generated?*
D. *Why does such a relationship exist?*
E. *What is summation, incomplete and complete tetanus?*
F. *How do they occur?*
G. *Why is the contractile force greater in the case of tetanus than during a single twitch?*

This exercise not only provides a rather friendly and effortless way to understand the above problems, but the molecular mechanisms lying behind these phenomena will also be explained.

3.7.2. The structure of the program

The Ph.I.L.S. program has 4 main simulations from which you will use the "***Skeletal Muscle Function***". This simulation demonstrates how the skeletal muscle operates using 3 different subsimulations ("***Stimulus-Dependent Force Generation***", "***Length-Tension Relationship***" and "***Principles of Summation and Tetanus***"). You can start each of these by clicking on it with the left mouse button. The structure of each subsimulation is similar and easy to follow using the instructions given by the program. The screen appearing demonstrates the aim of the particular lab. Here (and everywhere from this point on) one can click on the items appearing in red. By doing so, an explanation of the relevant item is provided. Clicking on their top-right corner can close these windows. In the next section, the theoretical background of the present experiment is provided. Some test questions will also show up, aiding the proper understanding of the exercise. At the end of the review of the theory, the virtual experiment may be commenced. One should note that the theoretical information is always available by clicking on the "**Aim**" tag.
When the experimental set-up appears, it is highly recommended to go through the "**WETLAB**" section of the simulation, where it is explained how the preparation is performed in reality. The movies demonstrating the various parts of the preparation can be started by clicking on the highlighted items. When the video demonstration is finished, the virtual set-up may be turned on by clicking on the "**Power**" button of the data acquisition unit (DAU). (It is always worth reading through the information

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displayed at the bottom of the screen.) When the DAU is on, the cables should be attached as instructed (Just click on the plugs and drug them to the appropriate connectors of the DAU while the mouse button is pressed. By releasing the mouse button, the cable will be automatically attached.) After setting up the equipment, the experiment may start. To set the amplitude of the electrical stimulation just click on the upward arrowhead seen in the "Control panel". To apply the stimulation hit "**START**". The muscle shortens, and the force (or tension) generated is recorded. The tension is instantaneously plotted and demonstrated on the screen. In case of investigating the length-tension relationship, the initial length of the muscle will be changed. Therefore, one should click on the "**Zoom**" button, to allow the fine adjustments of the position and pre-stretch of the muscle. The amplitude of the mechanical force generated by the muscle can be measured by clicking on the top of the curve exhibiting the time-tension diagram. The relevant data show up in the "**DATA**" field, and these can be copied to the Exercise Book and transferred into the logbook by clicking on the "**write/open journal**" button (situated on the right of the "**ERASE**" and "**START**" buttons). Hitting this button opens a new window presenting the correlation between the stimulus intensity and the amplitude of the tension. A graphical representation of the data pairs is also provided. This window can be hidden by clicking on the "X" in the top-right corner, although it is not necessary. However, DO NOT PRESS "**FINISH**", as it will close the window permanently, and your data will be lost. The curves presented on the display can be erased any time by hitting "**ERASE**". When the chart is ready, press "**FINISH**", and a notepad will show up presenting a few questions related to the experiment. In some parts of the simulation program animations can be started by clicking on the "**Play**" symbol or on "**View Animation**". Animations can be stopped and restarted any time. The "Animation" presented in conjunction with the 4th question in the second part of the simulation is extremely helpful and the review provided here is essential to understand the molecular mechanism of the correlation between length and tension. Close every window and exit this part of the simulation by clicking on the "**MAIN MENU**" in the top line of the simulation screen.

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PRINCIPLE PARAMETERS IN PHYSIOLOGY

Body fluid compartments

Total body water (TBW):	60 % of body weight
Intracellular fluid:	60 % of TBW
Extracellular fluid (ECF):	40 % of TBW
Interstitial fluid:	47 % of ECF
Intravascular:	17 % of ECF

Composition of intracellular fluid

(only the most important cations, can be different among cell types and species)

Na^+	8 - 20 mmol/L
K^+	135 - 150 mmol/L
Ca^{2+}	0.1 - 1.0 μmol/L

Extracellular fluid compartment

Composition of human blood plasma: (mmol/L)

Na^+	133 - 146	Cl^-	99 - 111
K^+	3.5 - 5.3	HCO_3^-	22 - 31
Mg^{2+}	0.6 - 1.1	phosphate	0.80 - 1.45
ionized (free) Ca^{2+}	1.13 - 1.32	total Ca^{2+}	2.1 - 2.6

Osmolarity of human blood plasma:	275 - 301 mosmol/L
pH of human blood plasma:	7.38 - 7.42

Other components of human blood plasma:

glucose:	3.6 - 6.0 mmol/L
urea:	3.6 - 7.2 mmol/L
bilirubin:	3 - 17 μmol/L
protein:	60 - 80 g/L

Parameters of blood

Total blood volume:	80 mL/kg body weight
Hematocrit (Htc):	0.35 - 0.50
Sedimentation rate of RBC's:	< 10 mm/h
bleeding time:	2.5 - 9.5 min
platelets:	150 - 400 G/L

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Hemoglobin concentration in blood:	115 - 165 g/L
RBC count:	4.2 - 6.1 T/L
Mean corpuscular hemoglobin (MCH):	27 - 31 pg
Red cell index (RCI):	1
Mean corpuscular volume (MCV):	80 - 99 fL

White blood cell count (WBC):	4.8 - 10.8 G/L
Differential blood count:	
segmented neutrophils:	40 - 74 %
eosinofils:	0.1 - 5.0 %
basofils:	0.1 - 1.5 %
monocytes:	3.4 – 9.0 %
lymphocytes:	19 - 41 %

Gas transport in blood:

	combined with Hb	bicarbonate	dissolved
O_2	99 %		1 %
CO_2	5 %	90 %	5 %

Parameters of respiration

Pulmonary ventilation:	7 - 9 L/min (at rest)
Respiratory rate:	14 - 18/min (at rest)

Lung volumes:

tidal volume (TV):	0.5 L
inspiratory reserve volume (IRV):	2.5 L
exspiratory reserve volume (ERV):	1.0 L
vital capacity (VC):	4.0 L
residual volume (RV):	1.5 L
Tiffeneau-index (FEV_1/FEVC*100) :	> 80 %

Gas tensions: (mmHg)	pO_2	pCO_2
atmospheric air:	158	0.2
alveolar air:	100	40
venous blood:	40	46
arterial blood:	95	40

Parameters of the cardiovascular system

Cardiac output (at rest):	5.0 - 5.5 L/min
Maximal cardiac output during exercise:	20 - 25 L/min
Heart rate (at rest):	60 - 100/min
Stroke volume:	70 - 80 mL

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Parameters of the normal ECG:

P wave duration:	0.08 - 0.10 s
PQ interval:	0.12 - 0.20 s
QRS duration:	0.06 - 0.10 s
QT interval:	0.32 - 0.39 s

Hydrostatic pressure in systemic circulation: (mmHg)

left ventricle:	120/0
aorta:	120/80
capillary (arterial end):	30
capillary (venous end):	10
right atrium:	2 - 3

Hydrostatic pressure in pulmonary circulation: (mmHg)

right ventricle:	25/0
pulmonary artery:	25/8
pulmonary capillary:	7 - 8
left atrium:	6

Colloidosmotic pressure of blood plasma: 20 - 25 mmHg

Parameters of renal function

Urine production:	1 - 1.5 L/day
Density of urine:	1002 - 1030 g/L
pH of urine:	4.5 - 8.3
Density of isosmotic urine:	1010 - 1012 g/L

GFR:	125 mL/min
RBF:	1200 mL/min
RPF:	670 mL/min
ERPF=C_{PAH}:	600 mL/min
FF:	0.2
E_{PAH}:	0.9

Nutrition & metabolism

pH of gastric juice: 1-2

Basal metabolic rate (BMR): 155-175 kJ/h m^2

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Energy content of foodstuffs /caloric values/ (kJ/g):

	energy released by combustion in calorimeter	energy released by oxidation in the body
protein:	24	17
carbohydrate:	17	17
fat:	40	40

Oxygen energy equivalent: 19-21 kJ/L

Respiratory quotient (RQ):

- carbohydrate oxidized: 1
- fat oxidized: 0.7
- normal diet: 0.8 - 0.85

Daily recommended protein intake: 0.8 g/kg

Body mass index (BMI): 20-25 kg/m^2

Organs of sense

Spectrum of the visible light: 400-700 nm

Frequency range of hearing: 20-20000 Hz

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