UNIVERSITY OF DEBRECEN
FACULTY OF MEDICINE
DEPARTMENT OF PHYSIOLOGY

PHYSIOLOGICAL PRACTICES

for Dentistry Students

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TOPIC SHEET N^{o} 1

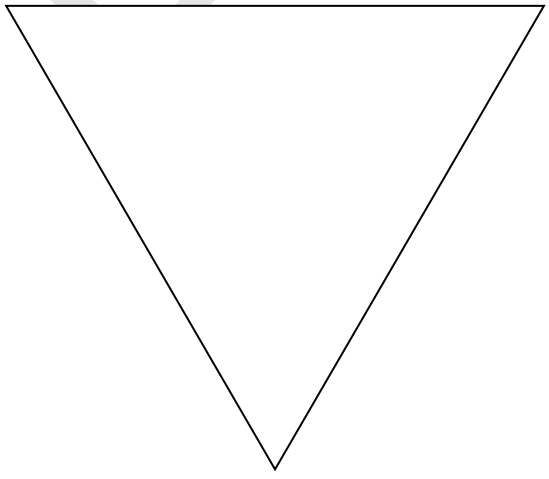
INVESTIGATION OF THE CARDIOVASCULAR FUNCTIONS

1.1. Make ECG recordings from two of your colleagues having different body constitutions using the standard bipolar leads (Lead I, II and III).

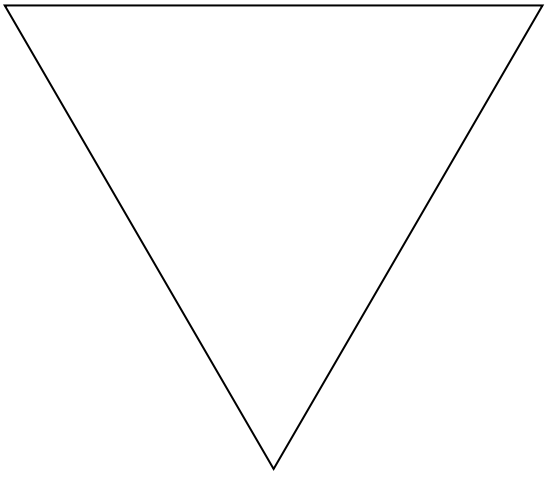
1.2. Evaluate the ECG recordings according to the criteria listed in the Practical guide!

Construct the R vector using the triangles below. Compare the two ECG recordings and summarize the most important differences. How would you explain your findings?

A)



B)



1.3. Make ECG recordings following a 15 second long hyperventilation, and following the Valsalva's and Müller's maneuvers. Summarize your findings.

1.4. Make ECG recordings after a period of moderate physical exercise (2 min, 50 W using the bicycle ergometer provided). Compare the ECG recordings obtained from both of your colleagues before and after the physical exercise; summarize the differences, and explain your findings.

1.5. Each member of the group should measure the blood pressure of one of his/her colleagues on both arms, and then indicate his/her own blood pressure.

1.6. Examine the pulse qualities determined on the radial artery of one of your colleagues, and summarize your findings. Repeat this investigation on the dorsal pedal and tibialis posterior arteries, and evaluate the differences if present.

1.7. Examine the heart sounds of one of your colleagues and summarize your findings. Determine the punctum maximum of each cardiac valve.

1.8. Listen to the heart sounds and murmurs recorded from patients suffering from aortic insufficiency and aortic stenosis. Summarize the most important findings, and make a scheme illustrating the relation between the heart sounds and the murmurs.

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The lab is completed:

…….. .....
date signature of lab teacher

TOPIC SHEET N° 2

DETERMINATION OF PARAMETERS CHARACTERISING THE RESPIRATORY FUNCTIONS

2.1. Determine the static and dynamic pulmonary parameters of two of your colleagues having different body compositions, before and after moderate physical exercise (bicycle ergometer; 2 min, 70 W). Indicate the appropriate values in the following tables.

Subject No.1

Static parameters	at rest	after exercise
Minute volume (MV; L/min)		
Tidal volume (TV; L)		
Respiratory frequency (RF; 1/min)		
Inspiratory reserve volume (IRV; L)		
Expiratory reserve volume (ERV; L)		
Vital capacity (IVC; L)		

Dynamic parameters (at rest)	Volume (L)	Tiffeneau's index
Forced expiratory vital capacity (FVC)		100%
Exhaled volume during the first half second of forced expiration (FEV*0.5)		FVC %:
Exhaled volume during the first second of forced expiration (FEV*1.0)		FVC %:
Exhaled volume during the first 6 seconds of forced expiration (FEV*6.0)		FVC %:
Forced inspiratory vital capacity (FIVC)		100%
Inhaled volume during the first second of forced inhalation (FIV*1.0)		FIVC %:

Peak inspiratory flow (PIF; L/s)	
Peak expiratory flow (PEF; L/s)	

Subject No.2

Static parameters	at rest	after exercise
Minute volume (MV; L/min)		
Tidal volume (TV; L)		
Respiratory frequency (RF; 1/min)		
Inspiratory reserve volume (IRV; L)		
Expiratory reserve volume (ERV; L)		
Vital capacity (IVC; L)		

Dynamic parameters (at rest)	Volume (L)	Tiffeneau's index
Forced expiratory vital capacity (FVC)		100%
Exhaled volume during the first half second of forced expiration (FEV*0.5)		FVC %:
Exhaled volume during the first second of forced expiration (FEV*1.0)		FVC %:
Exhaled volume during the first 6 seconds of forced expiration (FEV*6.0)		FVC %:
Forced inspiratory vital capacity (FIVC)		100%
Inhaled volume during the first second of forced inhalation (FIV*1.0)		FIVC %:

Peak inspiratory flow (PIF; L/s)	
Peak expiratory flow (PEF; L/s)	

Compare the resting respiratory values of the two volunteers, and then compare the respective parameters of each volunteer before and after physical exercise. Summarize and explain your findings.

2.2. Determine the metabolic rate of both subjects before and after physical exercise.

Subject No.1 Body surface: ……m^2

	at rest	after exercise
O_2 consumption (mL/min):		
Metabolic rate (kJ/h/m^2):		

Subject No.2 Body surface: ……m^2

	at rest	after exercise
O_2 consumption (mL/min):		
Metabolic rate (kJ/h/m^2):		

Compare the calculated values.

2.3. Using the available tubes with reduced diameter (simulating a situation with increased airway resistance), determine the dynamic respiratory parameters of one of your colleagues and summarize your findings.

Control

Dynamic parameters	Volume (L)	Tiffeneau's index
FVC		100 %
FEV*0.5		FVC %:
FEV*1.0		FVC %:
FEV*6.0		FVC %:
FIVC		100%
FIV*1.0		FIVC %:
PIF (L/s)		
PEF (L/s)		

With increased airway resistance

Dynamic parameters	Volume (L)	Tiffeneau's index
FVC		100 %
FEV*0.5		FVC %:
FEV*1.0		FVC %:
FEV*6.0		FVC %:
FIVC		100%
FIV*1.0		FIVC %:
PIF (L/s)		
PEF (L/s)		

Summarize the results, and make drawings of the respiratory “loop” graphs in the different cases. What pathological conditions may result in similar alterations?

2.4. Evaluate the combination of respiratory parameters provided by your tutor, and summarize your findings.

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The lab is completed:

…….. .....

date signature of lab teacher

TOPIC SHEET N^{o} 3

EXAMINATION OF THE CRANIAL NERVES

Generally, before starting the examination we should find time for inquiries. The examiner asks the subject whether he/she has previously noticed any change or dysfunction in the function to be tested, and what he/she thinks about his/her own performance. Record the anamnestic data in your topic sheets in all cases including the negative results as well. In the latter case you may write: "*anamnestic data indicating disorders were not mentioned*". In positive cases, however, you must give detailed description about the pathologic deviations.

3.1. Examination of the 1st cranial nerve

Examine the **olfactory functions** as it is described in the "LABORATORY GUIDE". Designate the odours tested, and compare the bilateral olfactory functions.

Anamnestic data:

	Name	left side	right side	difference
Odour I				
Odour II				
Odour III				
Odour IV				
Odour V				

Epicrisis:

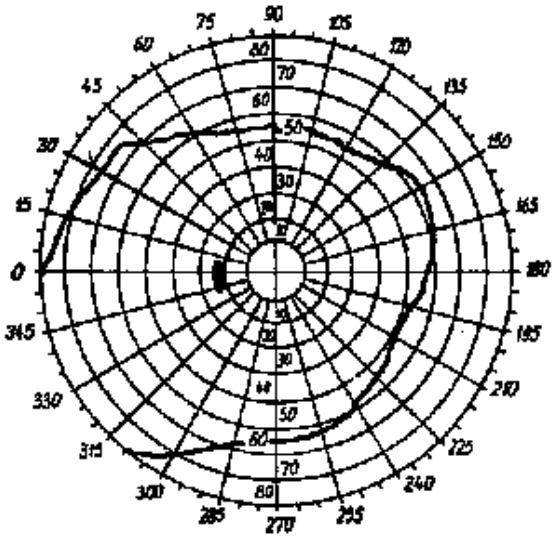
3.2. Examination of the 2nd cranial nerve

Anamnestic data (correction lens, scotoma or any visual disturbance):

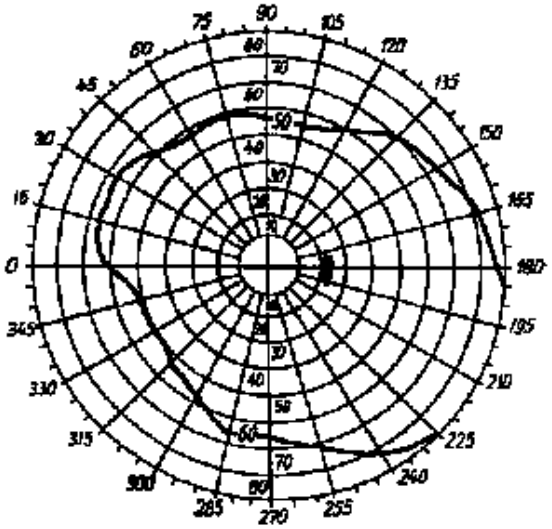
Determine the **visual acuity** of one of your colleagues. If your colleague wears glasses, the corrected visual acuity should be determined.

Visus of the left eye:
Visus of the right eye:

Determine the **visual field** of the same subject by **confrontal** examination and with **perimeter**. Outline the result on the following diagram. Localize the blind spot.

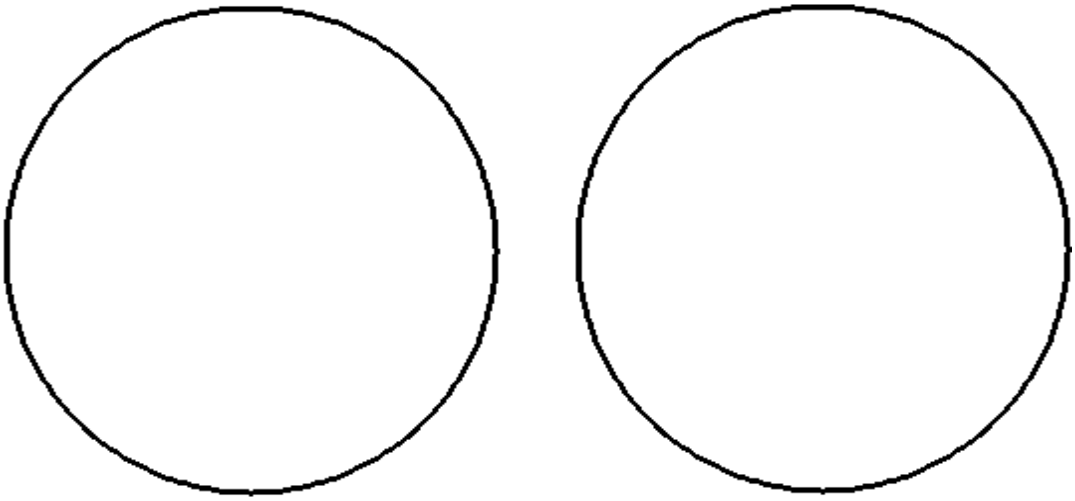


RIGHT EYE



LEFT EYE

Perform the **ophthalmoscopic examination** of the subject. Delineate the area around the fundus, then mark and characterize the components.



RIGHT EYE LEFT EYE

Epicrisis:

3.3. Examination of the 3rd, 4th and 6th cranial nerves

Examine the functions of the internal and external ocular muscles.

Anamnestic data (e.g. double vision):

Examine the spontaneous movements of the eyeballs at rest then repeat the examination following a 20-30 sec long rotation on a rotating chair.
List those functions which were altered by the rotation:

Epicrisis:

3.4. Examination of the 5th cranial nerve

Examine the motor and sensory functions of the 5th cranial nerve.

Anamnestic data:

Epicrisis:

3.5. Examination of the 7th cranial nerve

Examine the motor and sensory functions of the 7th cranial nerve.

Anamnestic data:

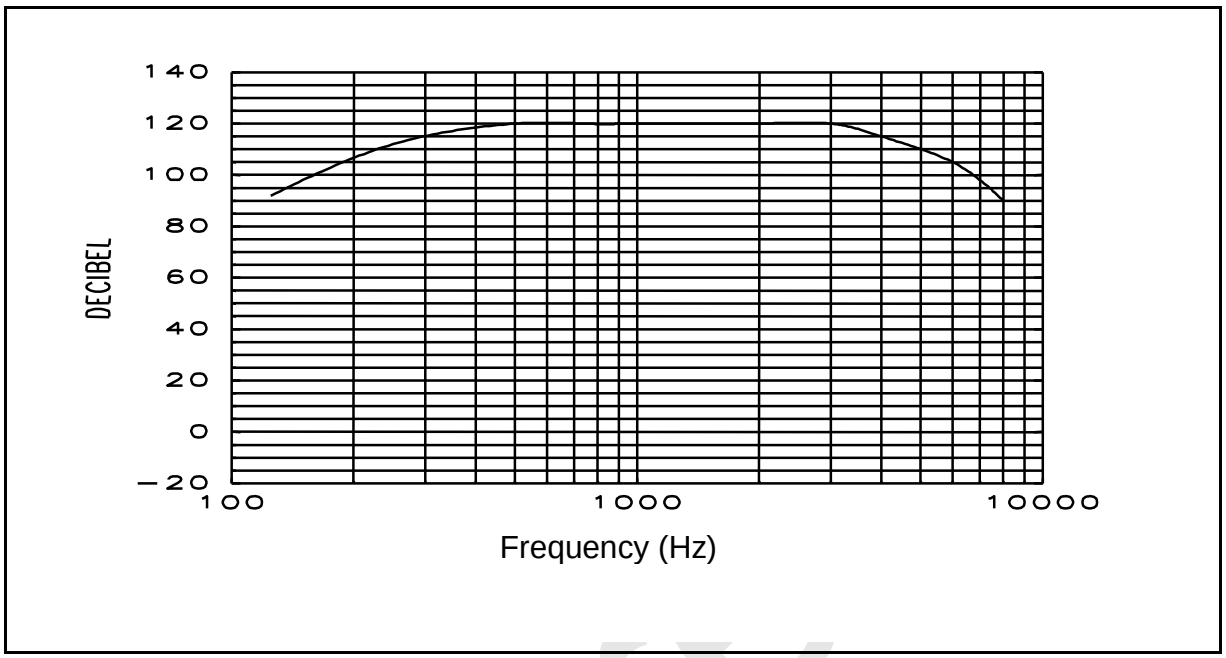
Epicrisis:

3.6. Examination of the 8th cranial nerve

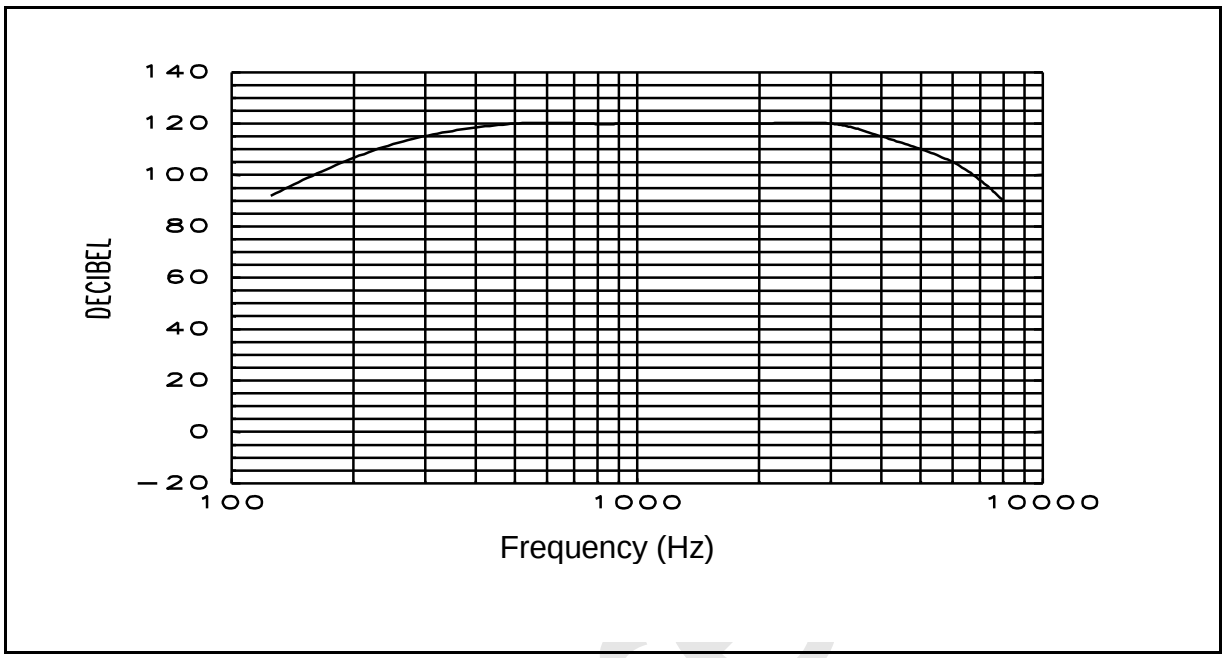
Examine the motor and sensory functions of the 8th cranial nerve.

Anamnestic data:

Perform the audiometric test on your colleague, examine the **air conduction** and plot the data obtained from both the left and the right ears in the same graph, using different symbols or colours.



Perform the audiometric test on your colleague, examine the **bone conduction** and plot the data obtained from both the left and the right ears in the same graph, using different symbols or colours.



Epicrisis:

The student was present:

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The lab is completed:

…….. .....
date signature of lab teacher

TOPIC SHEET N^{o} 4

EXAMINATION OF SOMATOSENSORY AND MOTORIC SYSTEMS

4.1. Examination of the 9th and 10th cranial nerves

Examine the sensory and motor functions of the cranial nerves listed above.

Anamnestic data:

Epicrisis:

4.2. Examination of the 11th cranial nerve

Examine the motor functions of the cranial nerve listed above.

Anamnestic data:

Epicrisis:

4.3. Examination of the 12th cranial nerve

Examine the motor functions of the cranial nerve listed above.

Anamnestic data:

Epicrisis:

4.4. Examine the motor functions of your colleague.

Anamnestic data:

Epicrisis:

4.5. Examine the sensory functions of your colleague.

Examine all sensory functions (superficial perception and dermolexia, depth perception, stereognosis)!

Anamnestic data:

Epicrisis:

4.6. Examine the reflex functions of your colleague.

Examine all reflex functions (stretch and superficial reflexes)!

Anamnestic data:

Epicrisis:

Rank the examined stretch reflexes by intensity (sensitivity).

\+ ..... > ..... > ..... > ..... > ..... > ..... -

4.7. Examine the coordination of your colleague.

Anamnestic data:

Epicrisis:

In the next step, examine the various elements of the coordination, then repeat the examination following a 20-30 sec long rotation on a rotating chair. Compare the functions prior to and after the rotation. List those functions which were altered by the rotation.

Functions altered by the rotation:

4.8. Determine your own **reaction time** using the appropriate computer program.

Determine the mean reaction time based on 5 subsequent trials. Write the results into the first section of the table below. Following this, keep on practicing for 3-5 minutes, then repeat the test, and write the results into the second section of the table.

Trial	Before training		After training	
	Sound	Light	Sound	Light
1.	ms	ms	ms	ms
2.	ms	ms	ms	ms
3.	ms	ms	ms	ms
4.	ms	ms	ms	ms
5.	ms	ms	ms	ms
Average	ms	ms	ms	ms

Compare the results obtained before and after training.

Shortening of the reaction time as the consequence of training
in case of light:..... ms
in case of sound:..... ms.

The student was present:

…….. ……..
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The lab is completed:

…….. .....
date signature of lab teacher

TOPIC SHEET N^{o} 5

COMPUTER AIDED ACQUISITION AND PROCESSING OF BIOLOGICAL SIGNALS

5.1. Connect the **transducer** to the input of the computer using the preamplifier interface unit. After starting the computer, start the data acquisition program with the **MEASURE** icon. Set slow (low frequency) data sampling (e.g. 100 point/second) and 300 s long data acquisition. Set the gain to **5** on the **preamplifier** unit.

Start the measurement by pressing **Start measurement** button. Set the baseline so that the imbalance LED is off on the preamplifier. Set the amplification so that the signal does not go out of scale even with the heaviest weight on. Place the calibration weights, one after the other, onto the arm of the force transducer. Mark the time of weight application on the computer by pressing the **Marker** button. A weight should be left hanging on the force transducer until a close to horizontal line is seen on the screen.

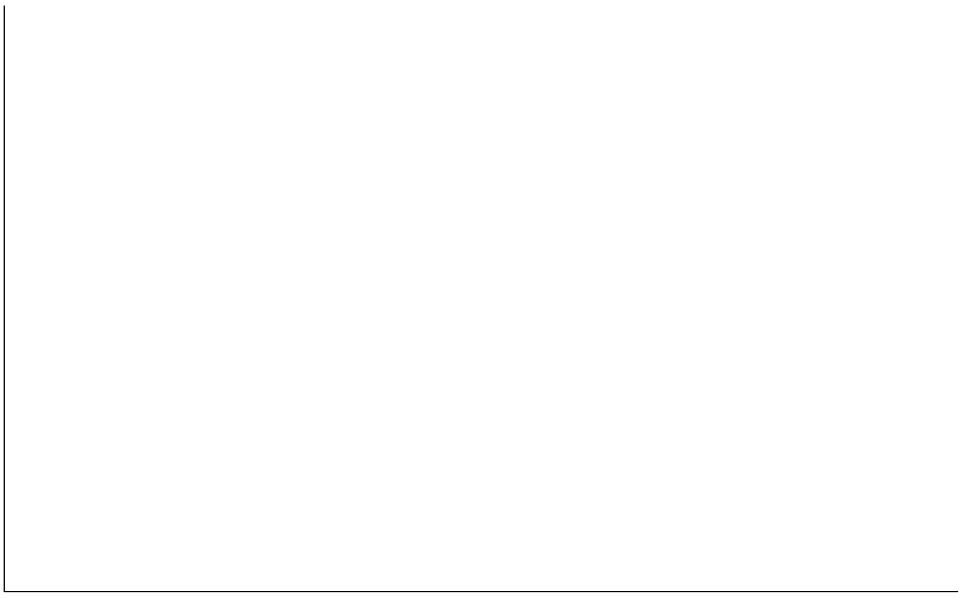
After completing the measurement, stop the data acquisition by pressing **Stop measurement** button. Save the data in a file (e.g. **CALIBRAT.DAT**). In the file name please use only letters and numbers without space or any extra symbol. You can change the time resolution or scroll the contents of the screen to left and right as it described in the Laboratory Practice User Guide. After saving the data file you can close the measurement program and start the data analysis by the **ANALYSIS** icon. Using the **left button of the mouse** you can select a part of the record with the green and red cursor lines. Certain parameters of the trace in the selected interval (time and measured value at start, time and measured value at the end) are displayed on the screen.

Fill in the table below by reading the amplitude (in Volts), of at least 5 points during the application of each weight. Calculate the average of these values. Enter the voltage measured in the absence of weights (baseline) into the first row.

The ID number of the force transducer to be calibrated: . . . . . .

Weight (g)	1st point (V)	2nd point (V)	3rd point (V)	4th point (V)	5th point (V)	Mean (V)
0						
1						
2						
5						
10						
20						
30						
50						

Plot the force as the function of the measured voltage.



Close temporary the data analyzer program to the Taskbar and start the program called **LINEFIT** for determining the slope of the calibration curve. First, enter the value of the baseline then the corresponding force and averaged voltage data from the table above. Finally enter a 0 and read the slope of the fitted straight line. Note that the program uses 9.81 m/s^2 for gravity acceleration.

The slope of the calibration line: . . . . . mN/V

Draw the calibration line onto the graph above.

5.2. Start the data analyzer program again from the Taskbar and read the **SAMPLE.DAT** data file into the program using Load data button (data input). You can select in the list of data files on common way. Set the previously determined calibration constant of the force transducer using. Note that although the conversion is automatically carried out by the program, the title of the Y-axis remains V (Volt).
Complete the following table for all contractions in the file. Increase the time resolution until only a single contraction is seen on the screen. Use the mouse to select the beginning and the end of the shortening. With the automatic analysis determine the characteristic parameters. The place of the maximum should be obtained by using the mouse and shift one of the pointers until you reach the maximum value given by the automatic analysis.

Parameters	1	2	3	4	5	6	7	8
Maximal rate of rise (s^{-1})								
Area under the curve (integral; mN•s)								
Time to peak (TTP; s)								
Half relaxation time (HRT; s)								
Value of maximum (mN)								
Position of maximum (s)								

Calculate the values representing the conditions before the first (before the 1st. marker), between the two (between the 1st and 2nd markers), and finally after the second solution change (after the 2nd marker) by averaging the corresponding data.

Parameters	Before the 1st marker	Between the 1st and 2nd markers	After the 2nd marker
Maximal rate of rise (s^{-1})			
Area under the curve (integral; mN•s)			
Time to peak (TTP; s)			
Half relaxation time (HRT; s)			
Value of maximum (mN)			
Average cycle length (s)			

Briefly summarize the effects of the solution changes.

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date signature of lab teacher

TOPIC SHEET N^{o} 6

STUDYING THE FUNCTION OF PERIPHERAL NERVES AND THE INNERVATED MUSCLES

6.1. Studying compound action potentials

Connect the **Signal generator** to the input of the computer. After starting the computer start the data acquisition program with the **MEASURE** icon.
Select the **Compound action potentials** option (1) on the Signal generator. Set the data acquisition frequency to 10 kHz, the duration of the recording to 2 s and the measuring range of ±1 V while selecting a trigger pulse with an amplitude of 5 V with the duration of 5 ms and with a repetition frequency of 1 Hz. Start the measurement by clicking on **Start measurement**. After finishing the measurement, save the record to a data file (e.g. **COMPAP**) with giving your name.

Draw a typical compound action potential.

Temporarily minimize the analyzing program to the Taskbar and start analyzing by clicking on the **ANALYZE** icon. Determine the characteristic parameters of the different nerve fiber types. For the calculation consider that the distance between the stimulating and measuring electrodes was 20 cm.

Action potentials	**1st**	**2nd**	**3rd**	**4th**	**5th**
Conduction velocity (m/s)					
Relative proportion (% of 1st)					

What causes the difference in the conduction velocity of the different fiber types?

6.2. Investigating receptor potentials

Select the **Tonic receptor potential** option (4) and then its corresponding **action potentials** (7) on the Signal generator. Start the data acquisition program from the Taskbar and set the data acquisition frequency to 5 kHz, the measurement duration to 10 s and the measuring range of ±1 V. Apply a trigger pulse with an amplitude of 5 V, duration of 5 ms and a repetition frequency of 1 Hz. Start the measurement. After finishing the measurement, save the record to a data file (e.g. **TONICREC**) with giving a name.

Draw both the receptor and the action potentials. Repeat the previous steps after selecting **Phasic/tonic** (3) and **Phasic receptor potential** (2) and their corresponding **action potential** (6 and 5, respectively) on the Signal generator.

receptor potential action potential

tonic receptor

phasic/tonic receptor

phasic receptor

Temporarily minimize the analyzing program to the Taskbar and start analyzing by opening the **ANALYZE** program from the Taskbar, too. Fill in the table below with the characteristic parameters of the receptor potentials (RP) and of the action potentials (AP). Calculate the frequency using 10 consecutive APs.

	tonic-receptor		**phasic/tonic - receptor**		**phasic-receptor**	
	start of pulse	**end of pulse**	**start of pulse**	**end of pulse**	**start of pulse**	**end of pulse**
Amplitude of RP						
Frequency of AP						

What correlation can you find between the amplitude of the receptor potential and the frequency of the action potentials?

6.3. Smooth muscle action potentials

Select the **Smooth muscle action potentials** option (8) on the Signal generator. Start the data acquisition program from the Taskbar and set the data acquisition frequency to 5 kHz, the measurement duration to 15 s and the measuring range of ±1 V. Apply a trigger pulse with an amplitude of 5 V, duration of 5 ms and a repetition frequency of 1 Hz. Start the measurement. After finishing the measurement, save the record to a data file (e.g. **SMOOTHAP**) with giving a name.

Draw the smooth muscle action potential.

Temporarily minimize the analyzing program to the Taskbar and start analyzing by opening the **ANALYZE** from the Taskbar, too. Determine the characteristic parameters of the potential changes considering that the signals were recorded using a 10 times gain.

slow wave		action potential	
frequency	**amplitude**	**frequency**	**amplitude**

6.4. Tetanus on fast and on slow muscles

Select the **Tetanus** option (9 in fast and 10 in slow muscles) on the Signal generator. Start the data acquisition program from the Taskbar and set the data acquisition frequency to 5 kHz, the measurement duration to 12 s and the measuring range of ±1 V. Apply a trigger pulse with an amplitude of 5 V, duration of 5 ms and a repetition frequency of 1 Hz. Start the measurement. After finishing the measurement, save the record to a data file (e.g. **FASTMUSCLE**) with giving a name.

Draw the two curves corresponding to the two types of muscle. Draw the place of stimulations.

Close the measuring program and start analyzing by opening the **ANALYZE** program from the Taskbar. Determine the characteristic parameters of the tetanus.

	rate of rise	**time to peak**	**maximum**	**half relaxation time**
Fast muscle				
Slow muscle				

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The lab is completed:

…….. .....
date signature of lab teacher

TOPIC SHEET N^{o} 7.

EFFECTS OF ELECTROLYTES ON THE UTERINAL SMOOTH MUSCLE FUNCTION

Myometrium stripes isolated from adult female rats are used to study the effects of bivalent cations on the function of the uterus. After starting the computer start the data acquisition program with the **MEASURE** icon. Set the data acquisition frequency to 0.1 kHz, the duration of the recording to 1000 s and the measuring range of ±1 V.

Note. Drugs exert their effects relatively slowly (5-10 min) on smooth muscle contraction. In order to avoid disturbances in drug-actions, a minimum of 10 to 15 min washing period with drug-free Tyrode solution should be applied between the different drugs tested. During the washing period change the bath solution to fresh Tyrode solution in every two minutes.
Use the **Marker** button to mark the solution changes and washing out.
We suggest to measure and save all the effects of compounds asked by the practice sheet, then start analyzing and printing by clicking on the **ANALYZE** icon. Please do not forget to set the calibration constant of the force transducer, determined in Topic 8, before starting the data evaluation.

7.1. Record the spontaneous mechanical activity of the isolated uterine segment for 5-10 min.

RECORD:
File name: **Gain:**

7.2. Record the contractile pattern of the uterine stripe in Tyrode solution (containing 2.5 mM Ca^{2+}), then change the bath solution to **0.5 mM calcium containing Tyrode solution**. Record the effect of calcium reduction on uterine contraction then add a few drops of **calcium chloride solution** (0.1 M $CaCl_2$ stock solution) and continue recording until the spontaneous mechanical activity returns. Finally, switch again to normal Tyrode solution.

RECORD:

File name: **Gain:**

Measure the following parameters of the recorded smooth muscle contractions:

- cycle length of mechanical activity (CL)
- maximal force of contraction (F)
- time to peak tension (TTP)
- half-relaxation time (HRT)
- slope of rising phase of contraction (Slope)
- Integral

	CONTROL	0.5 mM Ca^{2+} TYRODE	After adding Ca^{2+}
CL (s)			
F (mN)			
TTP (s)			
HRT (s)			
Slope (mN/s)			
Integral (mN*s)			

7.3. After a washing period of 10 min, apply 50 µL of **magnesium chloride** solution from the 1 M stock. After recording the effect of magnesium, add 100-200 µL of calcium chloride from the 0.1 M $CaCl_2$ stock solution to the bath, and continue recording until the spontaneous mechanical activity returns. Finally, switch to drug-free Tyrode solution.

RECORD:

File name: **Gain:**

Measure the following parameters of the recorded smooth muscle contractions:

maximal force of contraction (F)

time to peak tension (TTP)

half-relaxation time (HRT)

	CONTROL	Mg^{2+}	Mg^{2+} + Ca^{2+}	WASHOUT
F (mN)				
TTP (s)				
HRT (s)				

7.4. After the recovery of normal activity of the preparation, change the bath solution to **calcium free Tyrode solution**. Record the effect of low calcium solution on the uterine contraction then add 50-100 µL of **barium chloride** solution from the 0.1 M stock. Record the effect of barium then add 20 µL of **papaverine** solution (Papaverinum hydrochloricum ampoule, containing 40 mg/mL papaverine) to the bath. Continue recording until the effects of papaverine develop. Finally, return to normal Tyrode solution.

RECORD:

File name: **Gain:**

The student was present:

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date signature of lab teacher or helper

The lab is completed:

…….. .....

date signature of lab teach

TOPIC SHEET N° 8

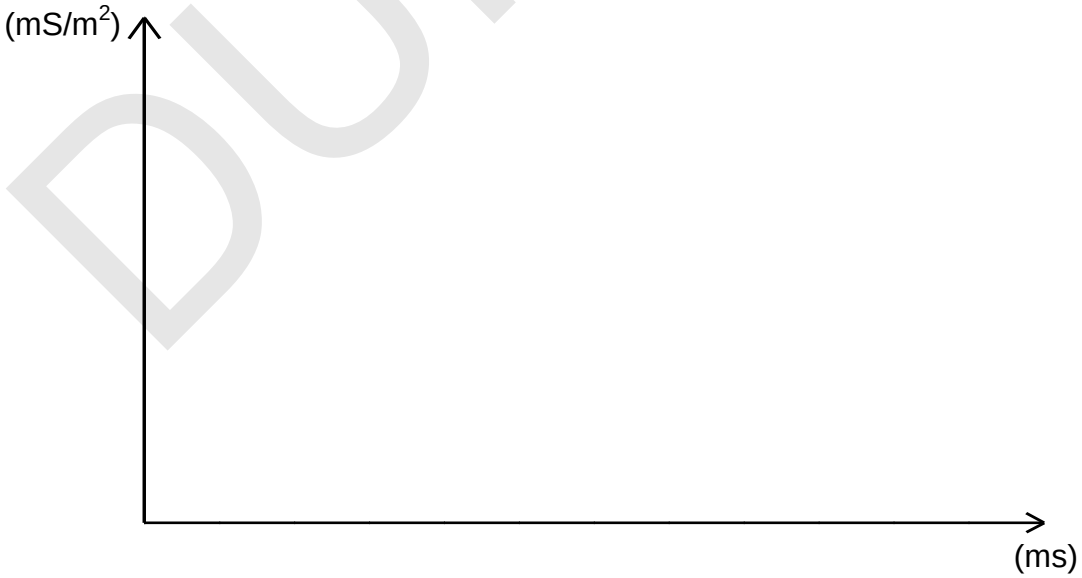
SIMULATION OF THE ACTION POTENTIAL IN THE SQUID AXON

8.1. Activation threshold of the cell membrane

Determine the activation threshold of the cell membrane to electrical stimulation at 0.1 ms pulse duration in the 9th point of the simulation program (S button in menu)! Draw the changes in the membrane potential and the conductances evoked by the threshold stimulus!

change in membrane potential:

change in conductances:



Amplitude of the threshold stimulus:.....($\mu A/cm^2$)

8.2. Temporal summation

Find the appropriate parameters of stimulation required to demonstrate temporal summation! Both electric pulses should be smaller than the threshold potential, and the second pulse should be even smaller than the first one. The duration of both stimuli should be 0.1 ms.

	1st pulse	2nd pulse
amplitude:	.....	.....
delay:	.....	

8.3. Refractoriness

Simulate the changes of excitability (refractoriness) following an action potential induced by a suprathreshold stimulus (duration: 0.1 ms)! Measure the shortest time required to restore the excitability to its resting level! Use longer simulation duration in display menu!

	1st pulse	2nd pulse
amplitude:	.....	.....

The resting level of excitability was restored at: **.....** ms, measured from the upstroke of the conditioning pulse.

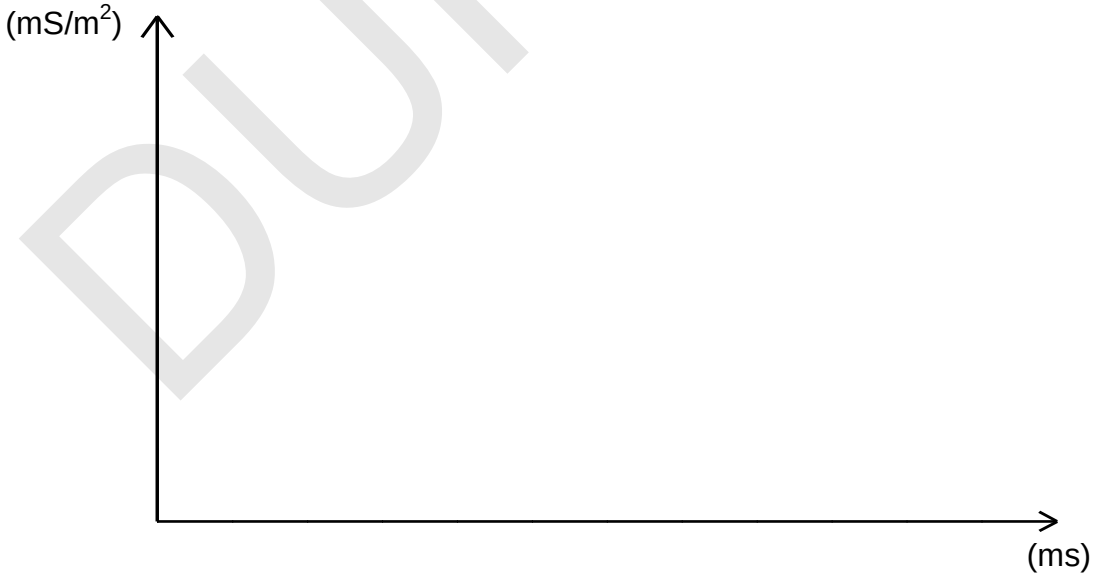
Find stimulatory parameters when the 2nd pulse has to be higher in amplitude than the 1st pulse itself! Determine the shortest time required for restoration of subnormal excitability!

	1st pulse	2nd pulse
amplitude:	.....	.....
duration:	.....	.....
shortest required time:	.....	

8.4. Depict curves representing potential and conductance changes evoked in the subnormal period!

potential changes:

conductance changes:



8.5. Effect of extracellular Na^+ concentration

Plot curves representing the changes in action potential configuration after changing (increased and decreased) the extracellular Na^+ concentration (Na^+ gradient in the Pharmacology menu)! Use 5 ms long timescale and high enough (150 $\mu A/cm^2$) stimulus amplitude.

Summarize the major differences observed!

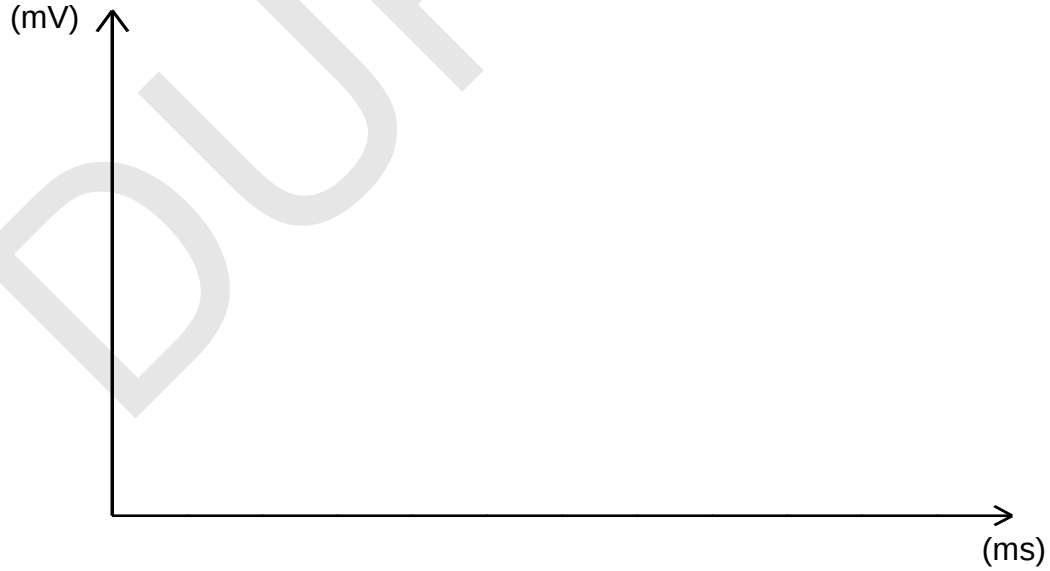
Change the relative extracellular Na^+ gradient between 0.1 and 2, measure the peaks of action potentials, and plot it. Use the Measure option in the Menu bar.

Na^+ gradient	Peak values of action potentials (mV)
0.1	
0.5	
0.7	
1.0	
1.2	
1.5	
1.9	

Summarize your observations!

8.6. Effect of extracellular K^+ concentration

Plot curves representing the changes in the spontaneous action potential generation after changing (increased and decreased) the extracellular K^+ concentration (K^+ gradient in the Pharmacology menu)! Use 100 ms long timescale and do not apply stimulation.



Summarize the major differences observed!

Change the relative extracellular K^+ concentration between 1 and 3, measure the frequency of the generated action potentials, and plot it.

K^+-gradient	Action potential frequency (1/s)
1.0	
1.5	
2.0	
2.5	
3.0	

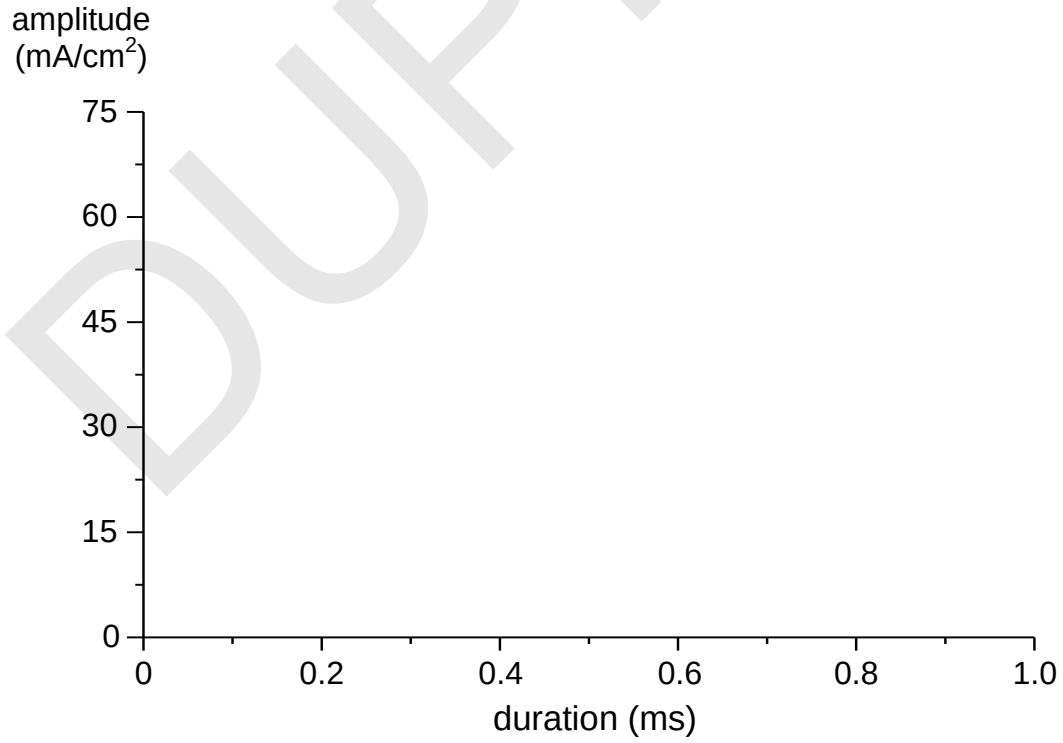
Summarize the major differences observed!

8.7. Construct the **strength-duration curve** on the model membrane using various combinations of pulse durations and the corresponding amplitudes required to reach the threshold intensity!

Parameters of stimuli applied

duration:	amplitude:
0.1 ms	.....mA/cm^2
0.2 ms	.....mA/cm^2
0.3 ms	.....mA/cm^2
0.4 ms	.....mA/cm^2
0.5 ms	.....mA/cm^2
0.6 ms	.....mA/cm^2
0.7 ms	.....mA/cm^2
0.8 ms	.....mA/cm^2
0.9 ms	.....mA/cm^2
1.0 ms	.....mA/cm^2

Plot the amplitude as the function of the duration of the stimulus!



8.8. Effects of different drugs on the action potential morphology

8.8.1. Plot the changes of the peak values of action potentials in the presence of the sodium channel blocker, saxitoxin (STX). Apply a 0.25 ms long stimulus of 100 μA/cm^2 for the experiment.

Concentration of STX	AP peak values
0 nM	……
0.5 nM	……
1.0 nM	……
1.5 nM	……
2.0 nM	……
2.5 nM	……
3.0 nM	……
3.5 nM	……
4.0 nM	……
4.5 nM	……
5.0 nM	……

Summarize the major differences observed!

8.8.2. Plot the changes of the action potential duration (APD) in the presence of the potassium channel blocker, tetraethyl-ammonium (TEA).

Concentration of TEA	APD
0 mM	……
1 mM	……
2 mM	……
4 mM	……
6 mM	……
10 mM	……
20 mM	……

Summarize the major differences observed!

8.8.3. Determine the frequency of the spontaneous action potential firing when TEA is applied in different concentrations. Use 100 ms long timescale and do not apply stimulation.

Concentration of TEA	Frequency (1/s)
0 mM	……
0.5 mM	……
0.6 mM	……
1 mM	……
5 mM	……
10 mM	……

Summarize the major differences observed!

The student was present:

…….. ……..
date signature of lab teacher or helper

The lab is completed:

…….. .....
date signature of lab teacher

TOPIC SHEET N^{o} 9

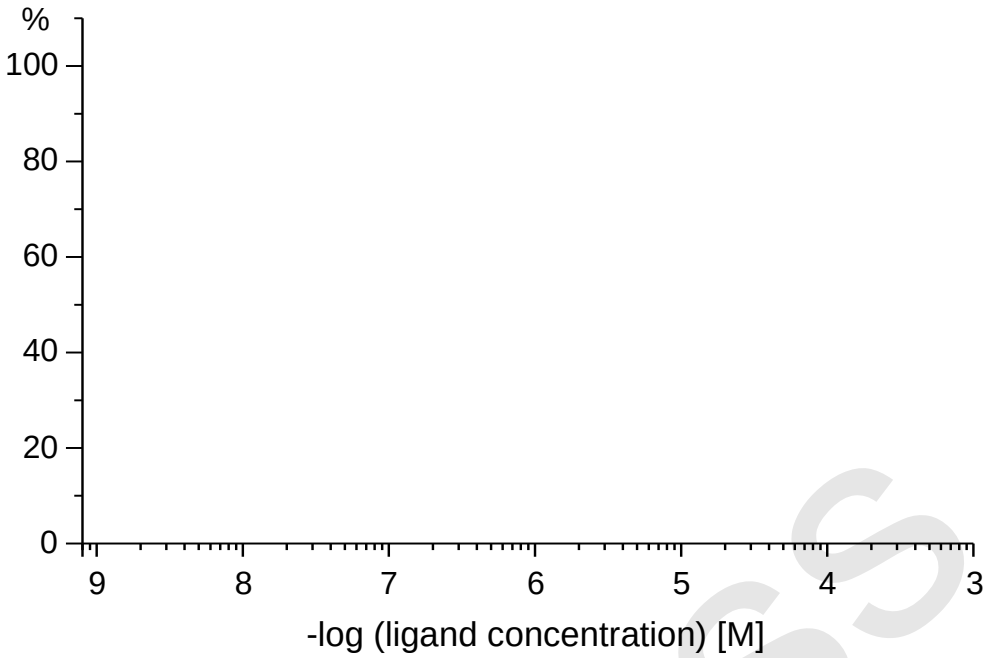
COMPUTER SIMULATION OF THE HUMORAL REGULATION OF THE INTESTINAL SMOOTH MUSCLE

9.1. Determine the concentration-dependence of the effects of acetylcholine on the mechanical properties of the ileum loop (dose-response curve). Please, use the concentrations suggested in the table below. Start the determination with washing out the unknown antagonist (21). Plot the relative tension of the ileum (after normalizing all values to the obtained maximum) as a function of the acetylcholine concentration and determine the half-effective concentration of the drug (EC_{50}).

In the following step determine the dose-response curve of acetylcholine (using the same acetylcholine concentrations) in the presence 0.05 µM atropine. Plot the dose-response curve with the new data.

Repeat the experiment after replacing atropine with 0.05 µM hexamethonium. Please, do not forget to wash the preparation between the application of the antagonists.

ACh concentration (µmol/L)	Change in tension in **Control**		Change in tension in the presence of **0.05 µM atropine**		Change in tension in the presence of **0.05 µM hexamethonium**	
	Measured value	Normalized to the measured maximum	Measured value	Normalized to the measured maximum	Measured value	Normalized to the measured maximum
0.001						
0.01						
0.05						
0.1						
1						
10						
100						
500						



Answer the following questions.

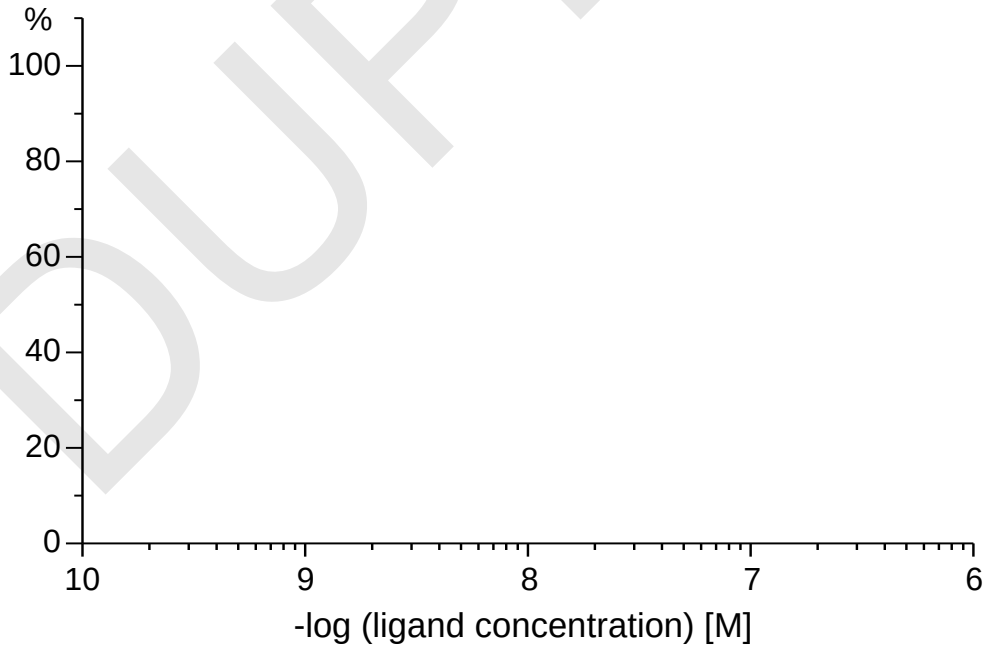
How do you explain the effect of acetylcholine? What kind of receptor is involved in the action of acetylcholine, and what second messenger pathway it is linked to?

What is the explanation of the effect of atropine? How and why did the EC_{50} of acetylcholine change in the presence of atropine?

How do you explain the data obtained in the presence of hexamethonium? Which receptors are inhibited by this antagonist, and where are they?

9.2. Using a new preparation study the concentration dependent effects of **atropine** on the response evoked by 0.3 μM acetylcholine. Use the table and concentrations provided below.

Atropine concentration (μmol/L)	Change in tension	
	Measured value	Normalized to the measured maximum
0		
0.0003		
0.001		
0.003		
0.01		
0.03		
0.1		
0.3		
1.0		

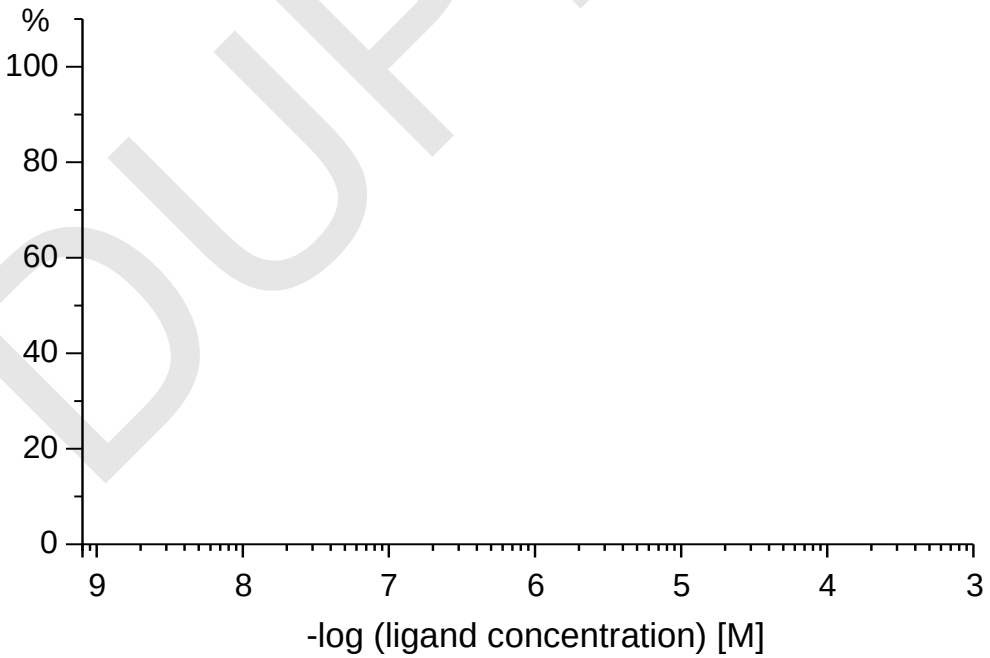


What is the half inhibitory concentration (IC_{50}) of atropine? Compare the affinities of the receptor for acetylcholine and atropine.

9.3. Discard the previously used ileum loop and determine the dose-response curve of acetylcholine for the new preparation as well. Use the table and the suggested concentrations provided below.

In the following step repeat the experiment in the presence of 0.5 µM physostigmine. Plot both sets of data.

ACh concentration (µmol/L)	Change in tension in **Control**		Change in tension in the presence of **0.5 µM physostigmine**	
	Measured value	Normalized to the measured maximum	Measured value	Normalized to the measured maximum
0.001				
0.01				
0.05				
0.1				
1				
10				
100				
500				

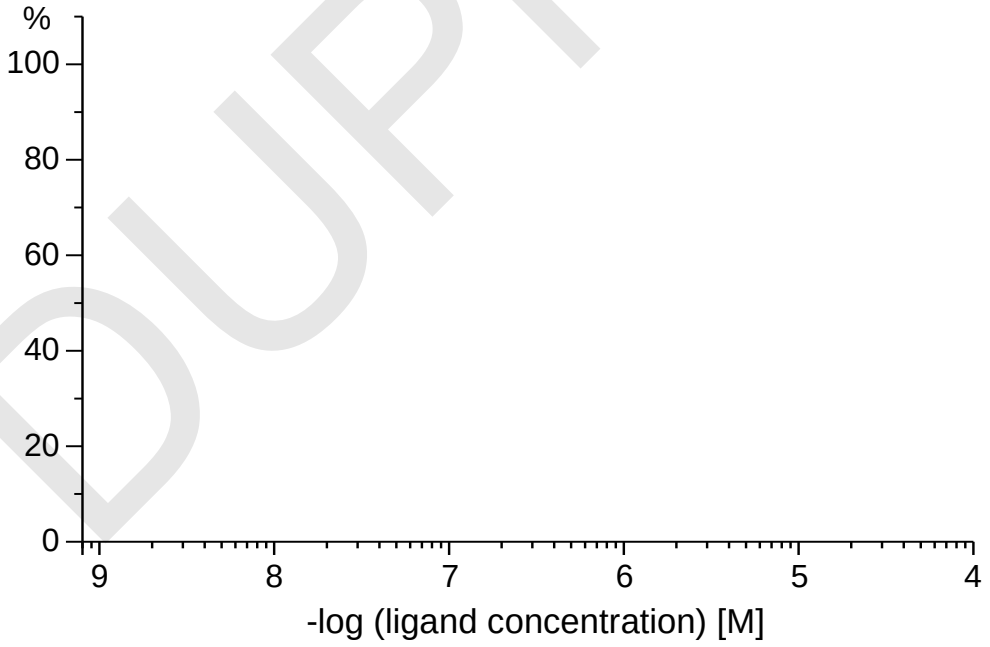


How do you explain the effect of physostigmine?

9.4. Use a new preparation to study the concentration dependent effects of **histamine** on the smooth muscle contractility. Use the concentrations given in the table below.

In the next experiment investigate how the presence of atropine (0.05 μM) influences the effects of histamine. Plot the obtained data.

Histamine concentration (μmol/L)	Change in tension in **Control**		Change in tension in the presence of **0.05 μM atropine**	
	Measured value	Normalized to the measured maximum	Measured value	Normalized to the measured maximum
0.001				
0.01				
0.05				
0.1				
1				
10				
100				

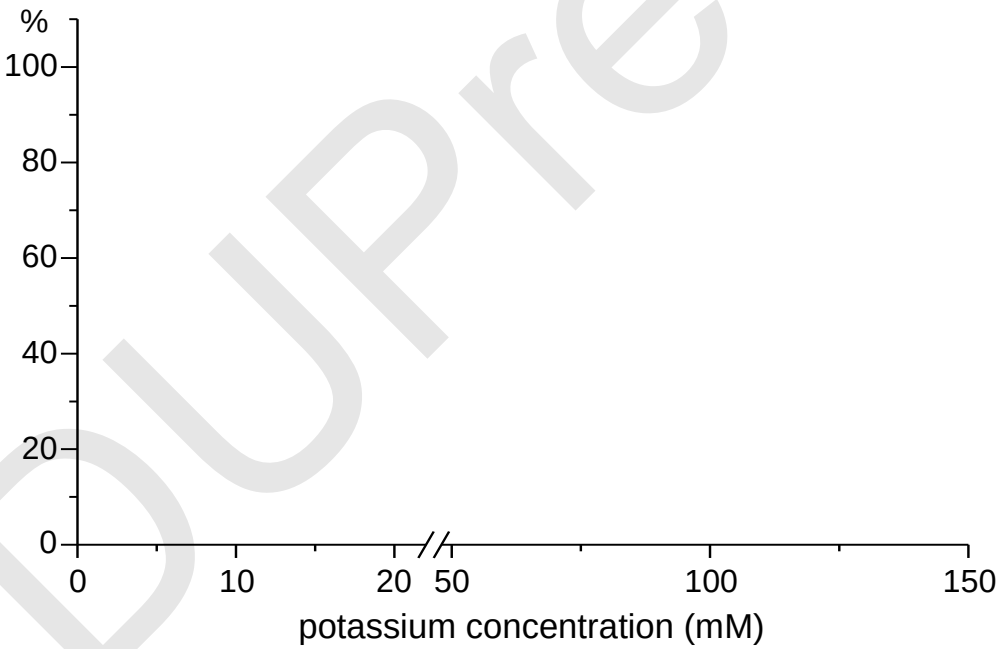


How do you explain the effect of histamine?

What do the data obtained in the presence of atropine suggest?

9.5. Discard the previously used ileum loop and study what happens to the smooth muscle activity if you increase the extracellular concentration of K^+. Plot the obtained data and answer the question.

Extracellular K^+-concentration (mM)	Change in tension	
	Measured value	Normalized to the measured maximum
5		
7		
10		
20		
50		
100		
150		



How do you explain the observed effects?

9.6. Investigate the effects of an “unknown” drug and try to identify it. Write a short report.

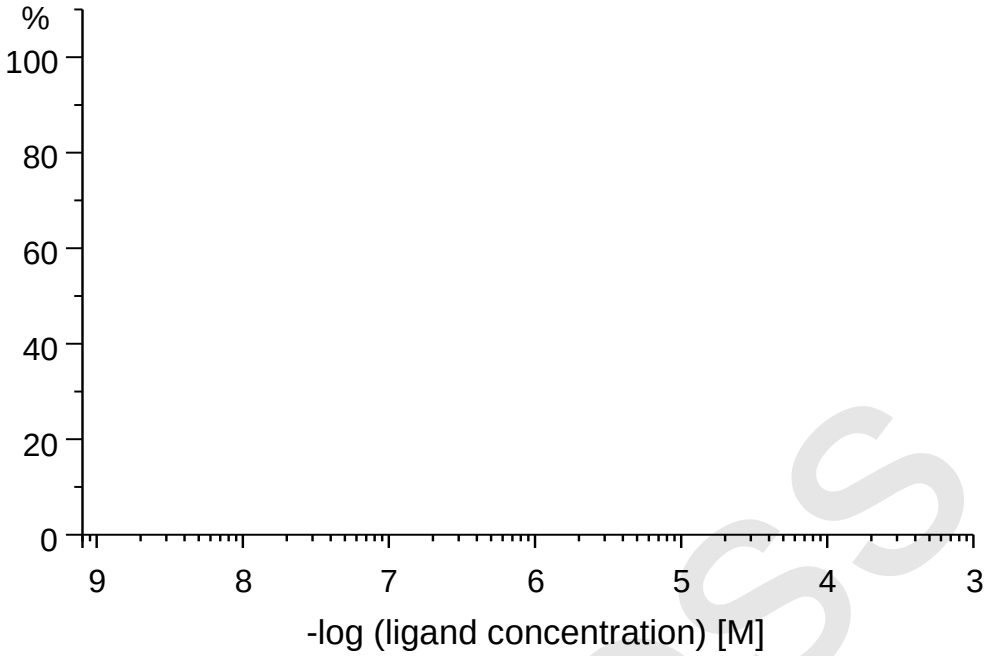
Working hypothesis:

Effects:

Results:

Ligand concentration (µmol/L)	Change in tension	
	Measured value	Normalized to the measured maximum

Dose-response curve:



Opinion:

The student was present:

…….. ……..

date signature of lab teacher or helper

The lab is completed:

…….. .....

date signature of lab teacher

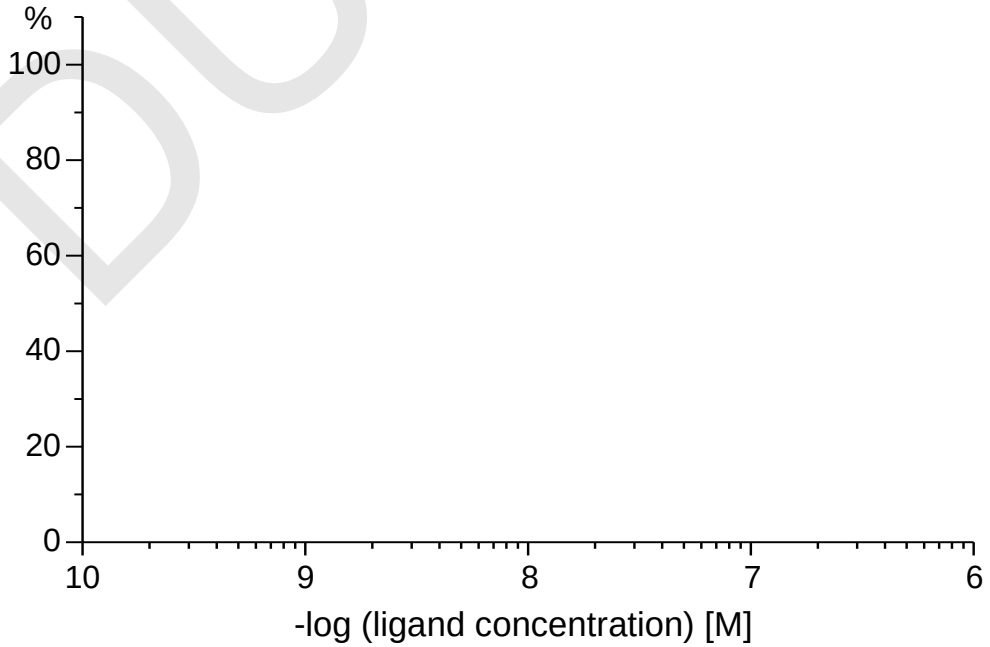
TOPIC SHEET N° 10

INVESTIGATION OF THE ENDOTHELIAL FUNCTION ON ISOLATED ARTERIAL RING

10.1. Determine the concentration dependent effects of norepinephrine on the mechanical properties of the arterial ring preparation with and without intact endothelium (dose-response curve). Use the concentrations suggested in the table below.

Plot the relative tension of both arterial rings (after **normalizing all values to the maximum measured using the endothelium free preparation**) as the function of the norepinephrine concentration and determine the half-effective concentrations of the drug (EC_{50}).

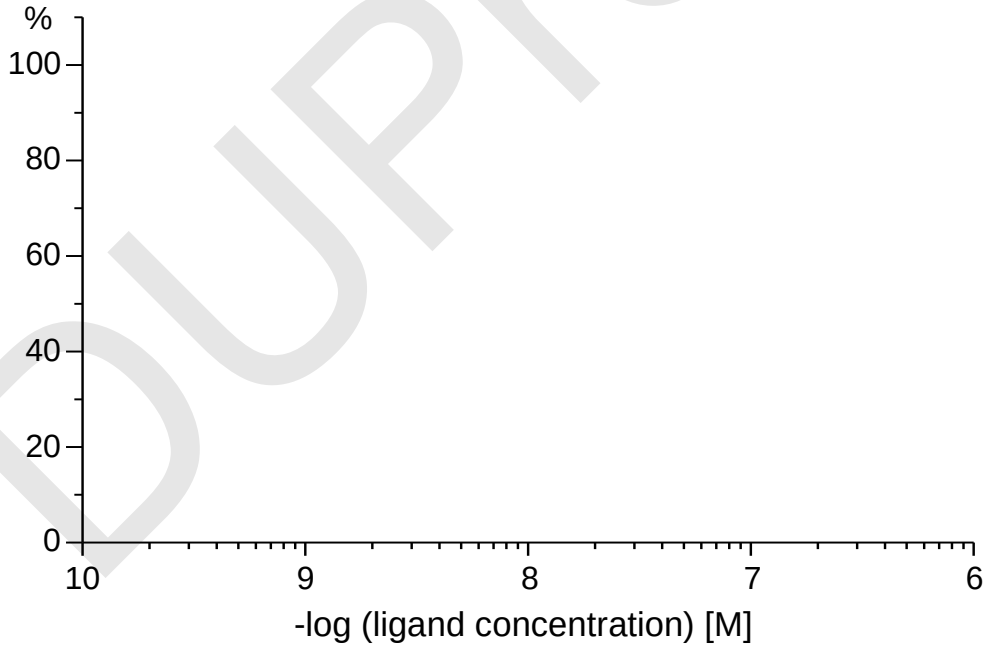
Norepinephrine concentration (mol/L)	**With intact endothelium**		**Without endothelium**	
	Measured value	Normalized to the measured maximum	Measured value	Normalized to the measured maximum
5×10^{-10}				
1×10^{-9}				
5×10^{-9}				
1×10^{-8}				
5×10^{-8}				
1×10^{-7}				
5×10^{-7}				
1×10^{-6}				



10.2. Repeat the experiments in the presence of 100 µmol/L L-NMMA (an NO-synthase inhibitor)!

Plot the relative tension of both arterial rings (after **normalizing all values to the maximum measured using the endothelium free preparation**) as the function of the norepinephrine concentration and determine the half-effective concentrations of the drug (EC_{50}). (Consider the change of tension induced by L-NMMA)

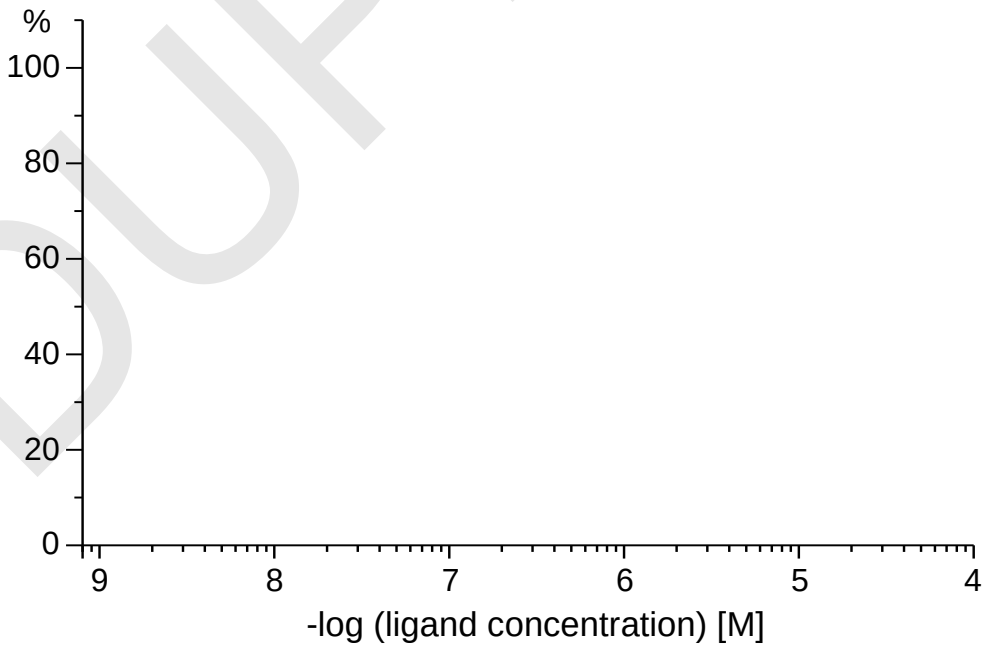
Norepinephrine concentration (mol/L)	With intact endothelium in the presence of **L-NMMA**		Without endothelium in the presence of **L-NMMA**	
	Measured value	Normalized to the measured maximum	Measured value	Normalized to the measured maximum
5×10^{-10}				
1×10^{-9}				
5×10^{-9}				
1×10^{-8}				
5×10^{-8}				
1×10^{-7}				
5×10^{-7}				
1×10^{-6}				



How do you explain the effect of NO synthase inhibition? What is the role of the endothelium in the modulation of the norepinephrine effect?

10.3. Determine and plot the concentration dependent effects of acetylcholine on the mechanical properties of both preparations. Since the arterial ring with intact endothelium does not show spontaneous activity, carry out the whole measurement in the presence of 5 x 10^{-7} mol/L norepinephrine. The endothelium free arterial ring preparation does not require norepinephrine to develop spontaneous tensions.

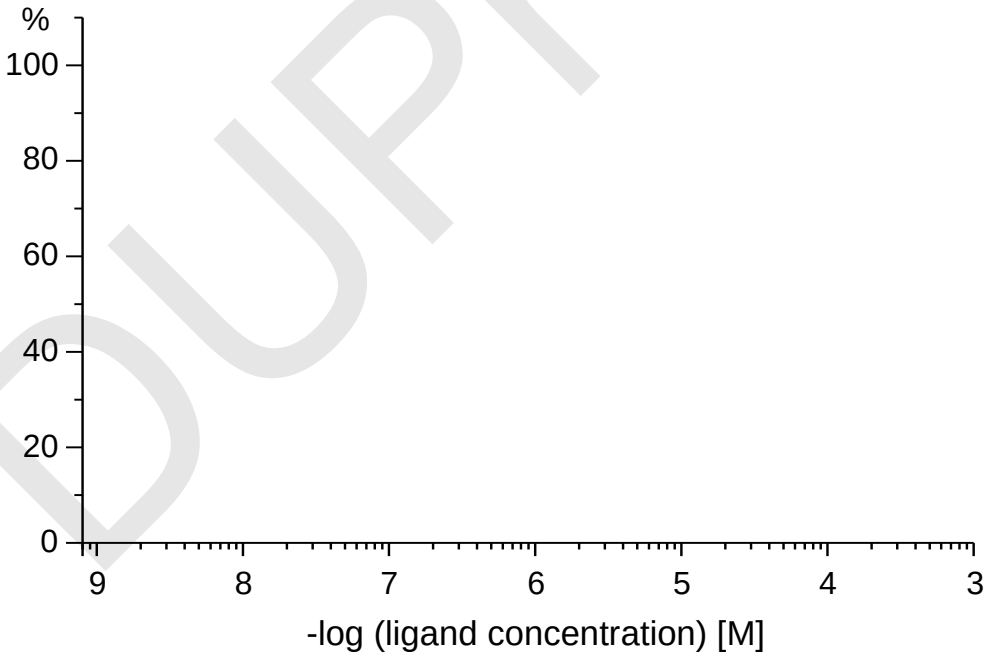
Acetylcholine concentration (mol/L)	With intact endothelium in the presence of **norepinephrine**		Without endothelium	
	Measured value	Normalized to the measured maximum	Measured value	Normalized to the measured maximum
0				
1 x 10^{-9}				
5 x 10^{-9}				
1 x 10^{-8}				
5 x 10^{-8}				
1 x 10^{-7}				
5 x 10^{-7}				
1 x 10^{-6}				
5 x 10^{-6}				
1 x 10^{-5}				
5 x 10^{-5}				
1 x 10^{-4}				



How do you explain the results?

10.4. Determine the type of cholinergic receptor present in the arterial ring preparation with intact endothelium. Create an experimental protocol for the investigation! Keep in mind that before the application of acetylcholine you have to apply an agonist (e.g. 5 x 10-7 mol/L norepinephrine) in order to activate the preparation! Select the proper activator from the list of agonists, and find the appropriate concentration. Use the table and graph below to record and show the results. Draw the dose response curve of acetylcholine obtained in the previous experiment in this graph in order to compare the data.

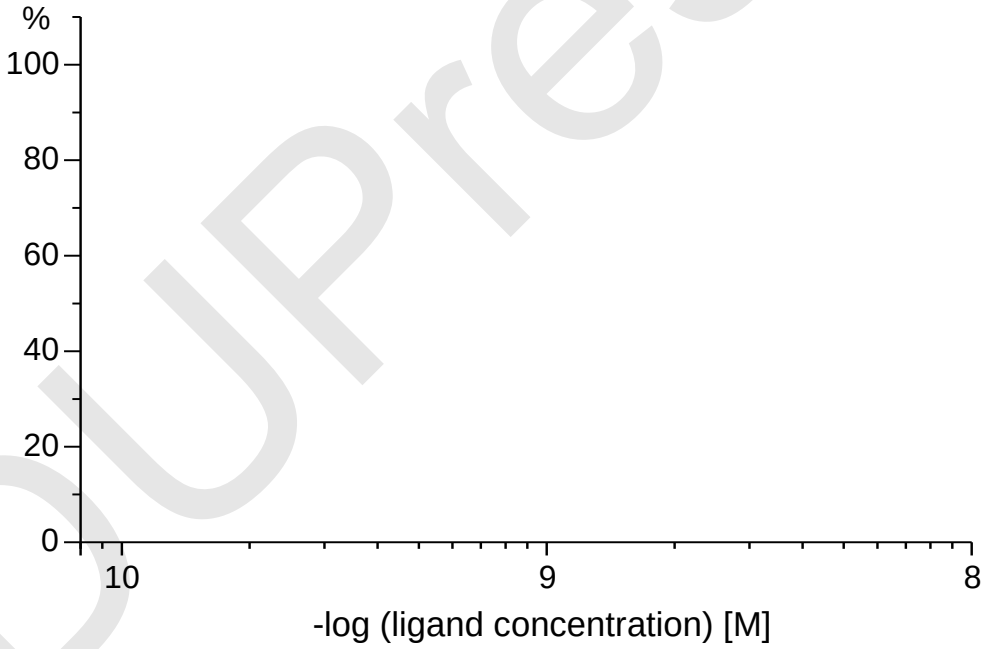
Acetylcholine concentration (mol/L)	Antagonist concentration		Antagonist concentration	
	Measured value	Normalized to the measured maximum	Measured value	Normalized to the measured maximum
0				



How do you explain the results?

10.5. Determine the concentration dependent effects of substance-P on both arterial rings. Before the application of substance-P, use norepinephrine to increase the basal tension of the preparations.

Substance-P concentration (mol/L)	With intact endothelium in the presence of **norepinephrine**		Without endothelium in the presence of **norepinephrine**	
	Measured value	Normalized to the measured maximum	Measured value	Normalized to the measured maximum
0				
1×10^{-10}				
3×10^{-10}				
5×10^{-10}				
1×10^{-9}				
3×10^{-9}				
5×10^{-9}				



How do you explain the results?

10.6. Investigate the effects of an “unknown” drug and try to identify it. Write a short report.

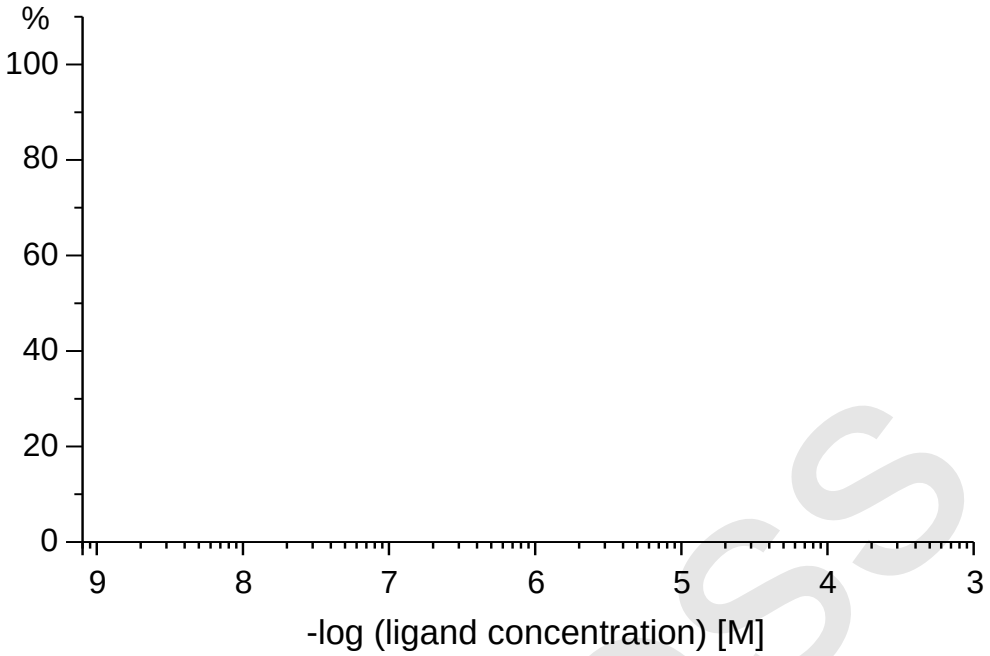
Working hypothesis:

Effects:

Results:

Ligand concentration (µmol/L)	Change in the tension	
	Measured value	Normalized to the measured maximum

Dose-response curve:



Opinion (do not forget to characterize the efficacy and potency of the drug):

The student was present:

…….. ……..

date signature of lab teacher or helper

The lab is completed:

…….. .....

date signature of lab teacher

TOPIC SHEET N^{o} 11

SIMULATION OF THE CARDIAC CYCLE AND THE STARLING MECHANISM

11.1. EVENTS OF THE CARDIAC CYCLE

11.1.1. Changes of the ventricular volume and pressure within one cardiac cycle

Examine the changes of the **ventricular volume, pressure** and **outflow** within one cardiac cycle in the left ventricle! Use high time resolution (0.5 s)! Plot the graphs and analyze them! Indicate the phases of ventricular filling, isovolumetric contraction, ejection and isovolumetric relaxation! What causes the ventricular ejection? How can you describe and interpret the pulse wave?

11.1.2. Relation of pressure and outflow in the ventricle and in the aorta

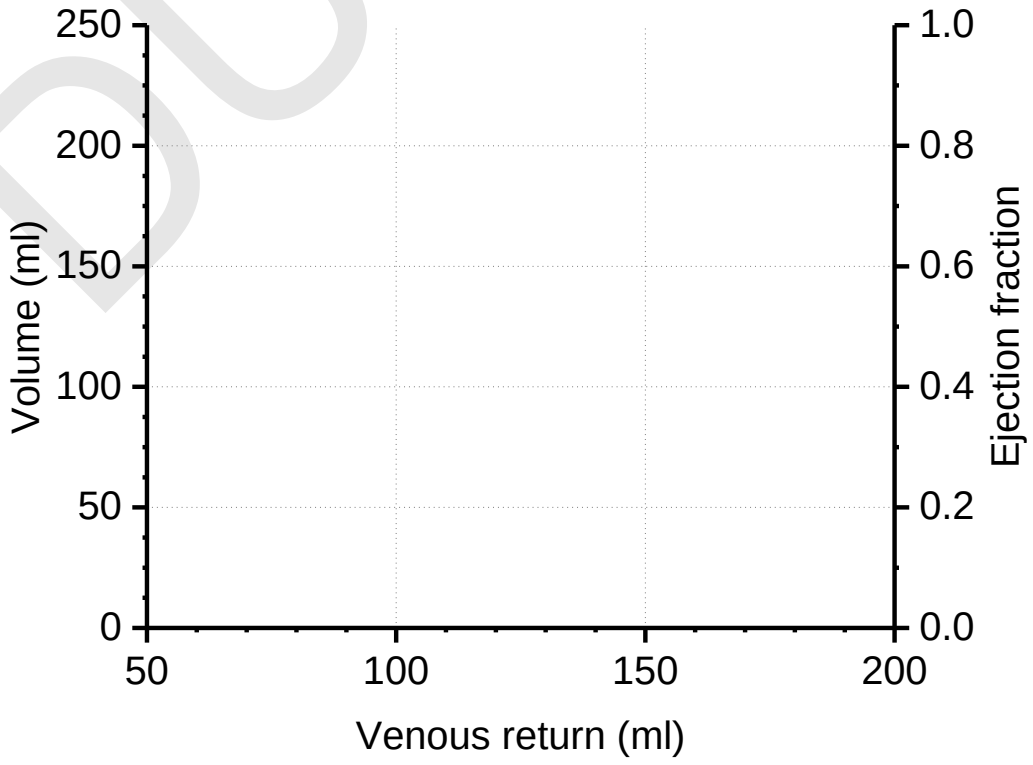
Draw and analyze how **pressure** and **outflow** changes in time within the **left ventricle** and within the **aorta**! Use maximal time resolution (0.5 s)! Indicate on the graphs where you expect sound effects to occur and explain these!

11.2. INTRINSIC ADAPTATION OF THE HEART TO DIFFERENT STATES OF CIRCULATION

11.2.1. Role of venous return

Demonstrate the effect of changing *venous return* on *end-diastolic* and *end-systolic volume*, *ejection fraction* and *systolic/diastolic aortic pressures* in the steady-state of a denervated heart! Use average *total peripheral resistance* (700 Hgmm*ms/ml) and *aortic elasticity* (1)! Plot the values of *ejection fraction, end-diastolic* and *end-systolic volume*!

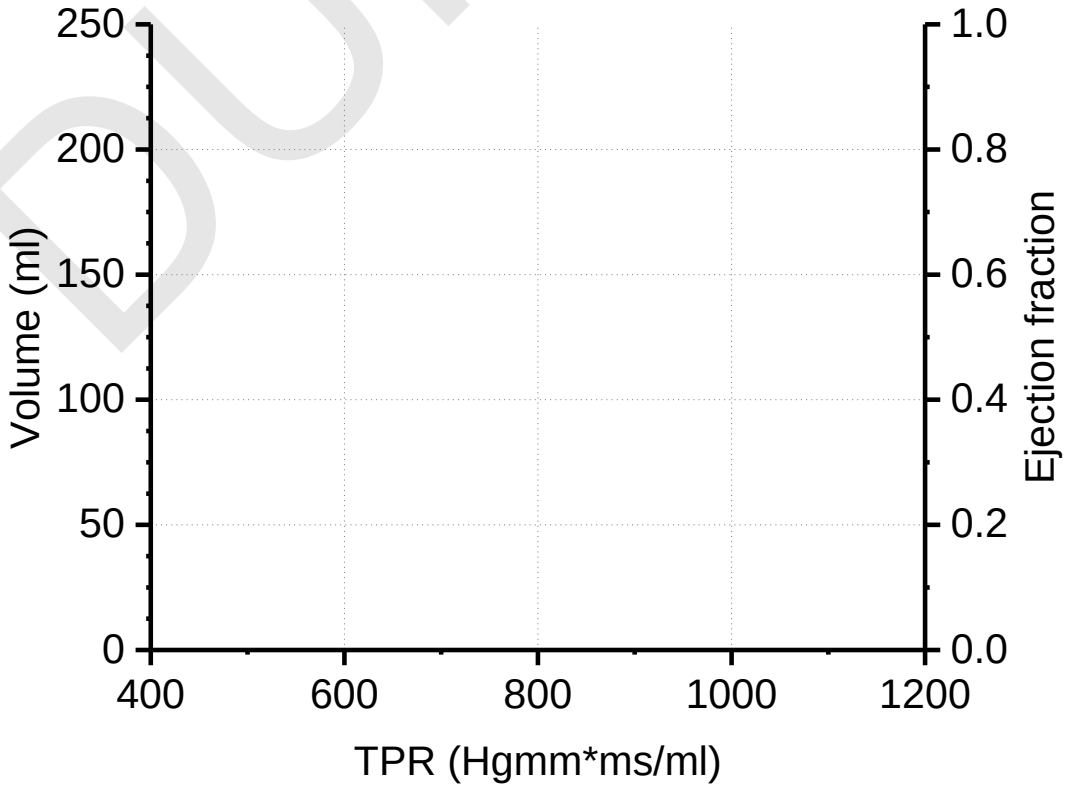
Venous return (ml)	End-diastolic volume (ml)	End-systolic volume (ml)	Stroke volume (ml)	Ejection fraction	Aortic pressure [sys/dia] (Hgmm)
60					/
80					/
100					/
120					/
140					/
160					/



11.2.2. Effect of peripheral resistance on end-diastolic volume

Demonstrate how changes in *total peripheral resistance (TPR)* affects *end-systolic* and *end-diastolic volume*, *ejection fraction* and *aortic pressures* in the steady-state of a denervated heart! Use average *venous return* (80 ml) and *aortic elasticity* (1) values! Plot the values of *ejection fraction, end-diastolic* and *end-systolic volume*!

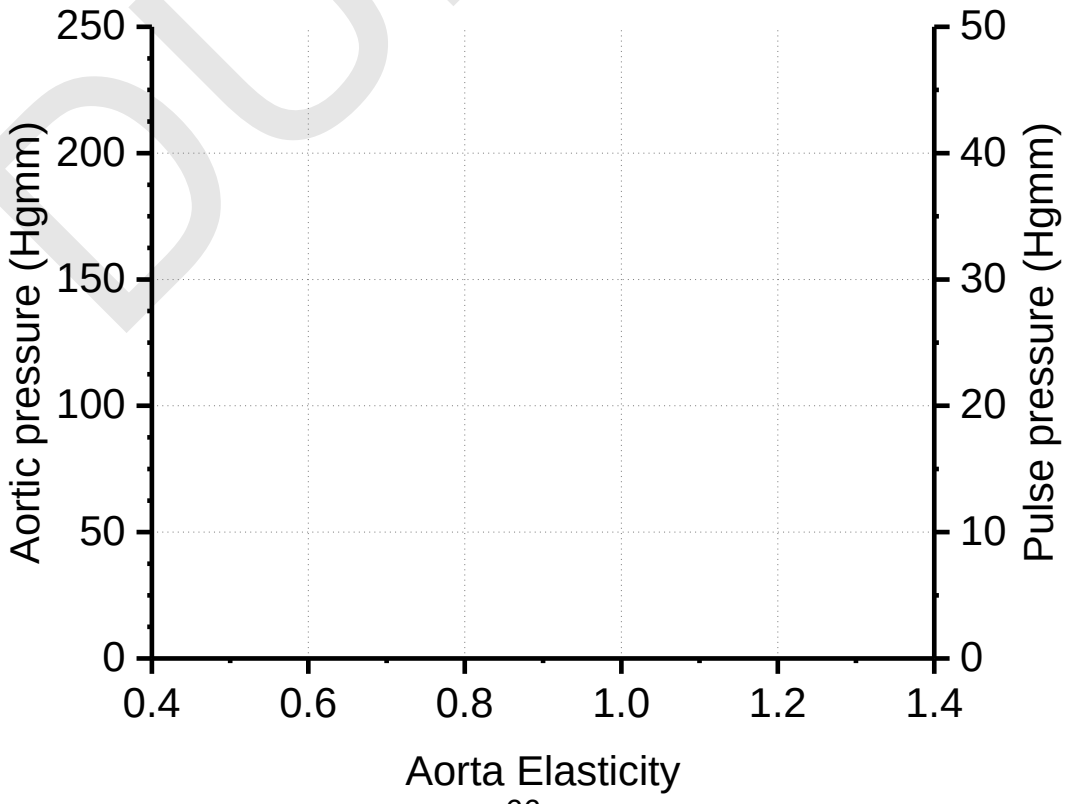
TPR (Hgmm *ms/ml)	End-diastolic volume (ml)	End-systolic volume (ml)	Stroke volume (ml)	Ejection fraction	Aortic pressure [sys/dia] (Hgmm)
500					/
600					/
700					/
800					/
900					/
1000					/
1100					/



11.2.3. Role of aortic elasticity

Demonstrate the effect of changing *aortic elasticity* on *diastolic and systolic pressures*, *ejection fraction* and *pulse pressure* in the steady-state of a denervated heart! Use average *total peripheral resistance* (700 Hgmm*ms/ml) and *venous return* (80 ml)! Plot the values of *systolic and diastolic* aortic pressure and *pulse pressure*!

Elasticity (Relative unit)	Diastolic pressure (Hgmm)	Systolic pressure (Hgmm)	Ejection fraction	Pulse pressure (Hgmm)
0.6				
0.7				
0.8				
0.9				
1				
1.1				
1.2				
1.3				



11.3. DYNAMICS OF THE STARLING MECHANISM

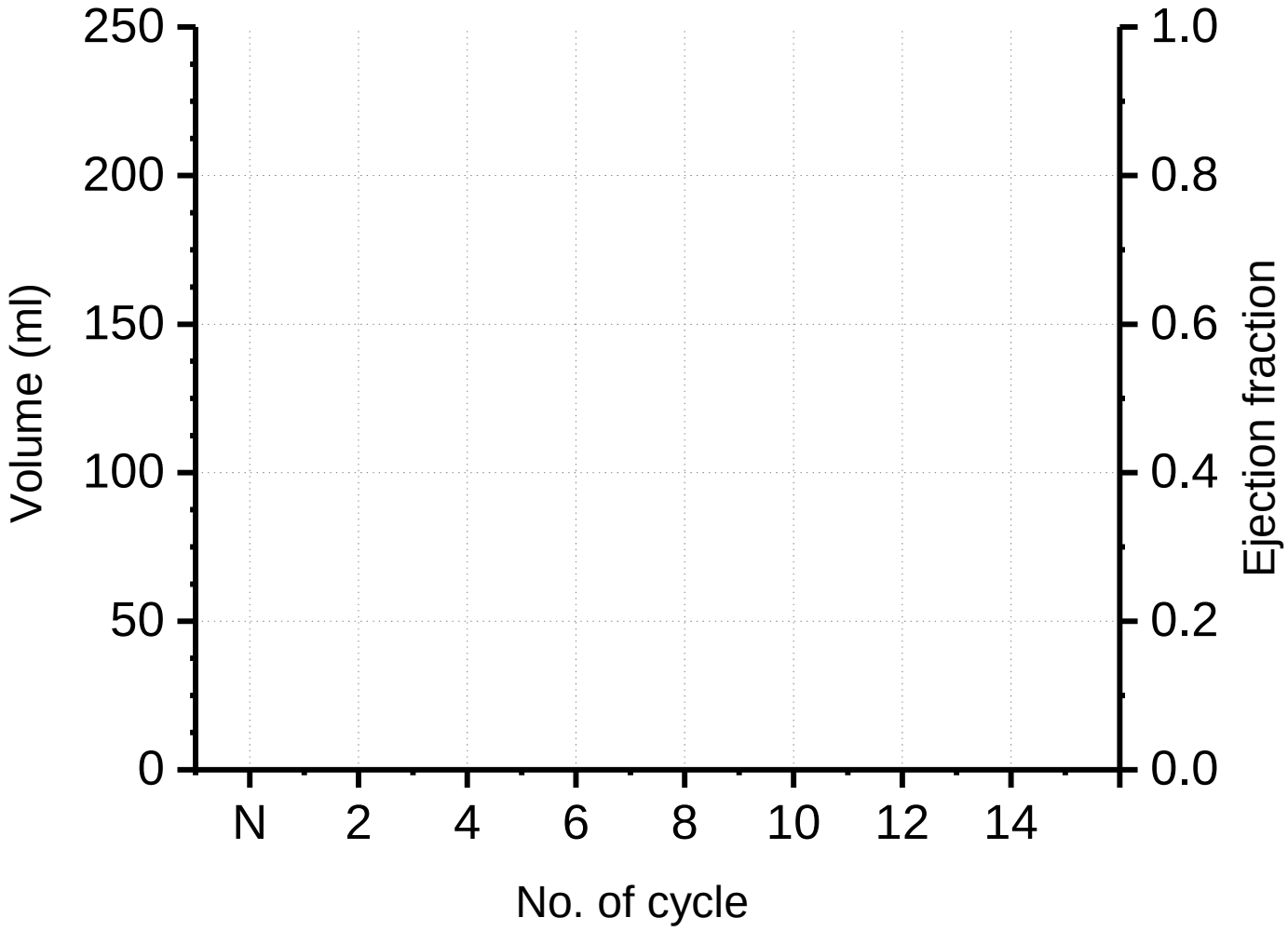
11.3.1. Role of increasing venous return

Demonstrate how a sudden large increase in *venous return* affects *end-diastolic and end-systolic volume*, *ejection fraction* and *systolic/diastolic aortic pressures* in a denervated heart! Use average *total peripheral resistance* (700 Hgmm*ms/ml) and *aortic elasticity* (1)! How many cardiac cycles are required for the new steady-state?

Original venous return: ..... ml Increased venous return: ..... ml

No. of cardiac cycle	End-diastolic volume (ml)	End-systolic volume (ml)	Stroke volume (ml)	Ejection fraction	Aortic pressure [sys/dia] (Hgmm)
original					/
After increased venous return					
1					/
2					/
3					/
4					/
5					/
6					/
7					/
8					/
9					/
10					/
11					/
12					/
13					/
14					/
15					/

Plot the values of *ejection fraction, end-diastolic* and *end-systolic volume*!



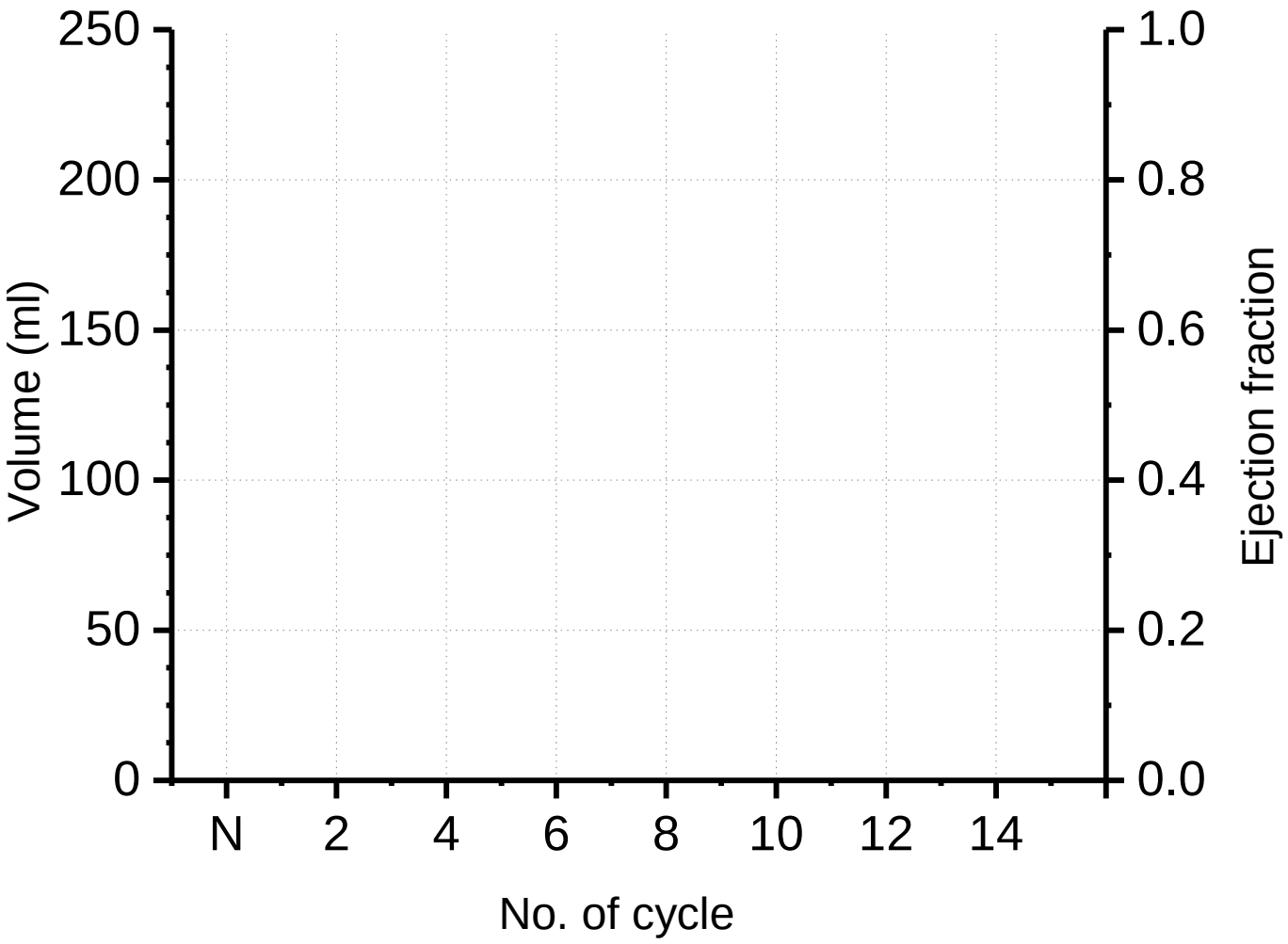
11.3.2. Role of increasing total peripheral resistance

Demonstrate how a sudden large increase in *total peripheral resistance (TPR)* affects *end-diastolic and end-systolic volume*, *ejection fraction* and *systolic/diastolic aortic pressures* in a denervated heart! Use average *venous return* (80 ml) and *aortic elasticity* (1)! How many cardiac cycles are required for the new steady-state?

Original TPR: ..... Hgmm*ms/ml Increased TPR: ..... Hgmm*ms/ml

No. of cardiac cycle	End-diastolic volume (ml)	End-systolic volume (ml)	Stroke volume (ml)	Ejection fraction	Aortic pressure [sys/dia] (Hgmm)
original					/
After increased TPR					
1					/
2					/
3					/
4					/
5					/
6					/
7					/
8					/
9					/
10					/
11					/
12					/
13					/
14					/
15					/

Plot the values of *ejection fraction, end-diastolic* and *end-systolic volume*!



The student was present:

…….. ……..
date signature of lab teacher or helper

The lab is completed:

…….. .....
date signature of lab teacher

CONTENTS