

UNIVERSITY OF DEBRECEN
FACULTY OF MEDICINE
DEPARTMENT OF PHYSIOLOGY

PHYSIOLOGICAL PRACTICES

for General Medicine Students



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PHYSIOLOGICAL PRACTICES

for General Medicine students

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Name:.....

Group:.....Semester:.....

The first semester is verified:

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date

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signature of tutor

Second semester PHYSIOLOGY LABS are verified:

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date

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Second semester NEUROPHYSIOLOGY LABS are verified:

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date

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TOPIC SHEET N° 1

INVESTIGATION OF THE CARDIOVASCULAR FUNCTIONS

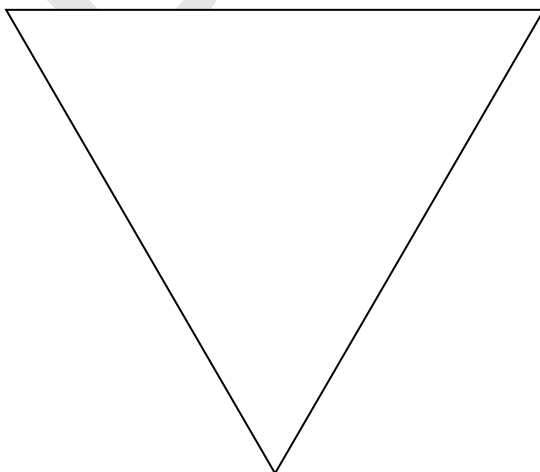
1.1. Make ECG recordings from two of your colleagues having different body constitutions using the standard bipolar leads (Lead I, II and III).

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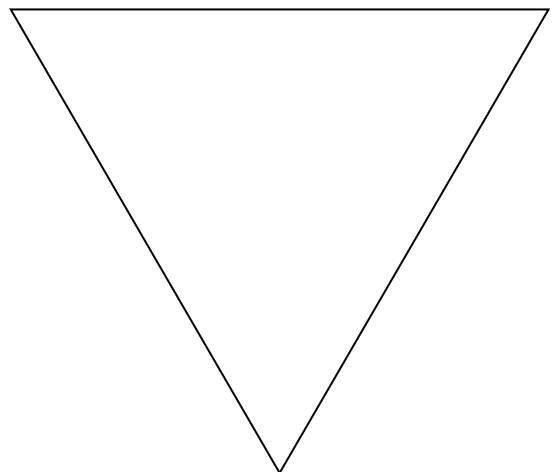
1.2. Evaluate the ECG recordings according to the criteria listed in the Practical guide!

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.

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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.

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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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date

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signature of lab teacher or helper

The lab is completed:

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date

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signature of lab teacher

TOPIC SHEET N° 2

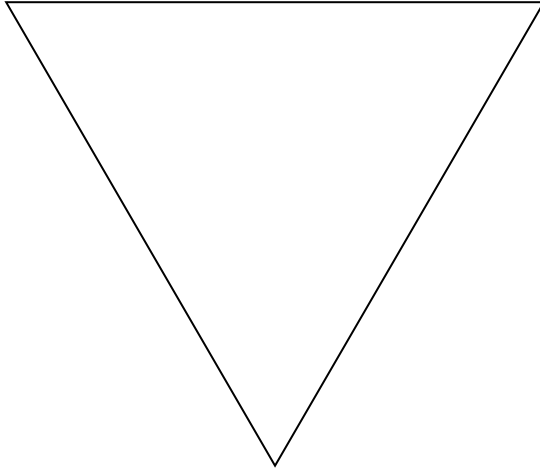
EVALUATION OF ECG RECORDINGS – RECOGNITION OF ECG ALTERATIONS

2.1. Evaluate two ECG recordings provided by your tutor, according to the criteria listed in the Practical guide. Make drawings of the characteristic parts of the recordings. What may cause such ECG alterations?

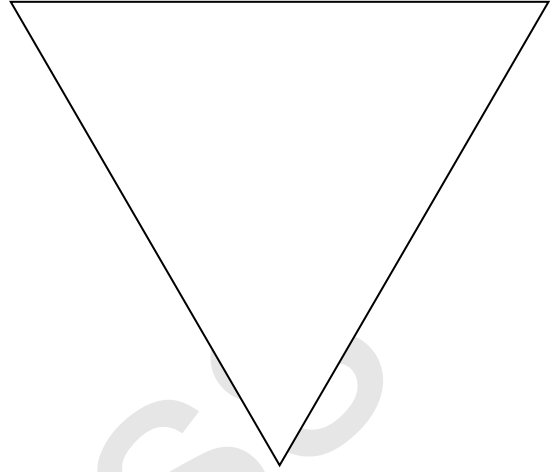
DUPress

2.2. Construct the R vector using the triangles below.

A)



B)



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date

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signature of lab teacher or helper

The lab is completed:

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date

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signature of lab teacher

TOPIC SHEET N° 3

DETERMINATION OF PARAMETERS CHARACTERISING THE RESPIRATORY FUNCTIONS

3.1. 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.

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

Subject No.1 Body surface:m²

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

Subject No.2 Body surface:m²

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

Compare the calculated values.

3.3. 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?

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

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The student was present:

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date

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signature of lab teacher or helper

The lab is completed:

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date

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signature of lab teacher

TOPIC SHEET N° 4

EFFECTS OF PHYSICAL EXERCISE ON THE CARDIORESPIRATORIC PARAMETERS. A STUDY OF RESTITUTION

4.1. Determine the following parameters of one of your fellow students at rest, and fill in the table below (“**REST**”). Ask the subject to perform a light **physical exercise (20 Watts, 1 minute;** on the bicycle ergometer), determine the same parameters at the end of the exercise, and fill in the “**TEST**” column).

Abbreviations	Parameters	REST	TEST (20 W)
P _S	Systolic blood pressure (mmHg)		
P _D	Diastolic blood pressure (mmHg)		
O ₂ Sat	Oxygen saturation (%)		
MABP	Mean arterial blood pressure (mmHg)		
HR	Heart rate (min ⁻¹)		
SV	Stroke volume (mL)		
CO	Cardiac output (L/min)		
SVR	Syst. vasc. resist. (dyn*sec*cm ⁻⁵)		
S	Ejection rate (ohm/sec)		
VET	Ventricular ejection time (ms)		
PEP	Preejection period (ms)		
QS2	Electromechanical syst. time (ms)		
DT	Diastolic period (ms)		

Are there any pathological phenomena on the ECG recordings, or subjectively from the patient that should be considered as contraindication of any further tests?

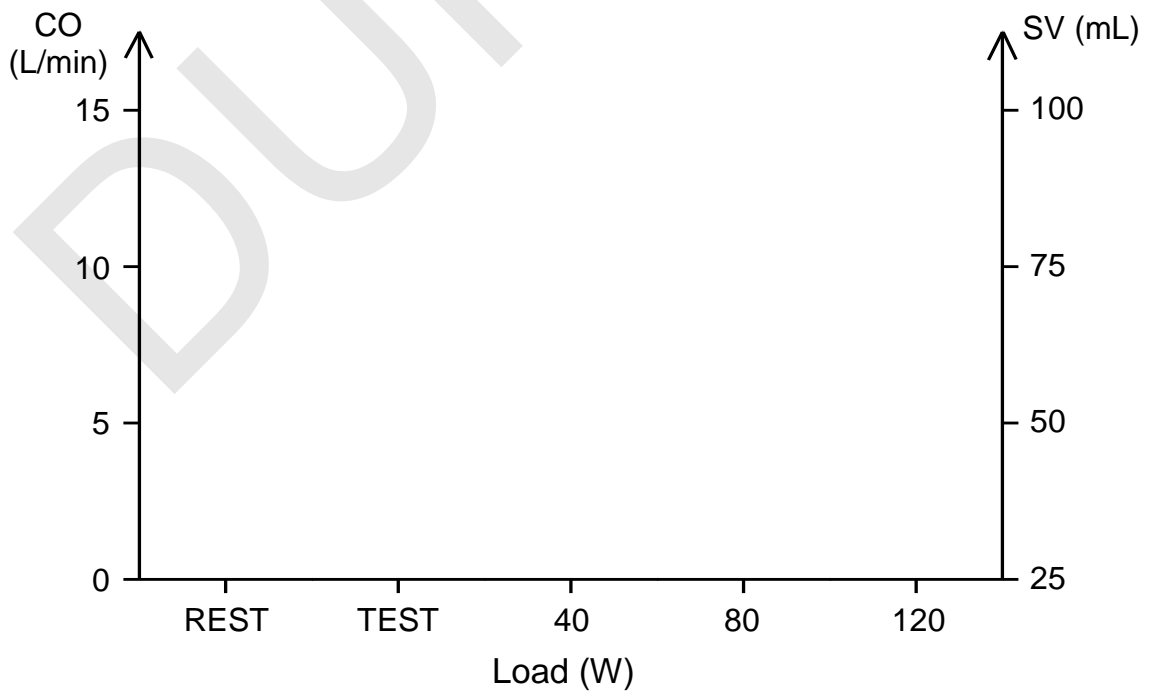
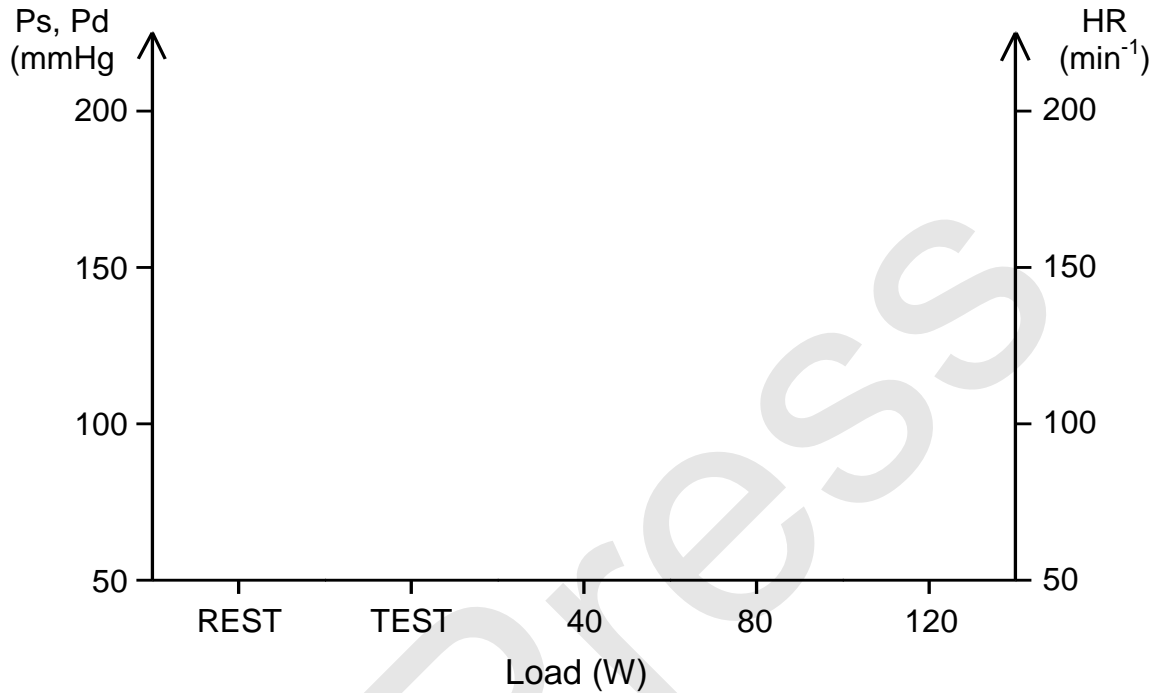
4.2. If no pathological phenomena are present, thus further tests are possible, continue the examination by increasing the load further, in **3 steps** (40 W, 80 W and 120 W; each load for 3 minutes with the bicycle ergometer). Describe the values of the cardiorespiratory parameters during each load, then study the restitution up to the initial values, and complete the following tables after each period of the physical exercise.

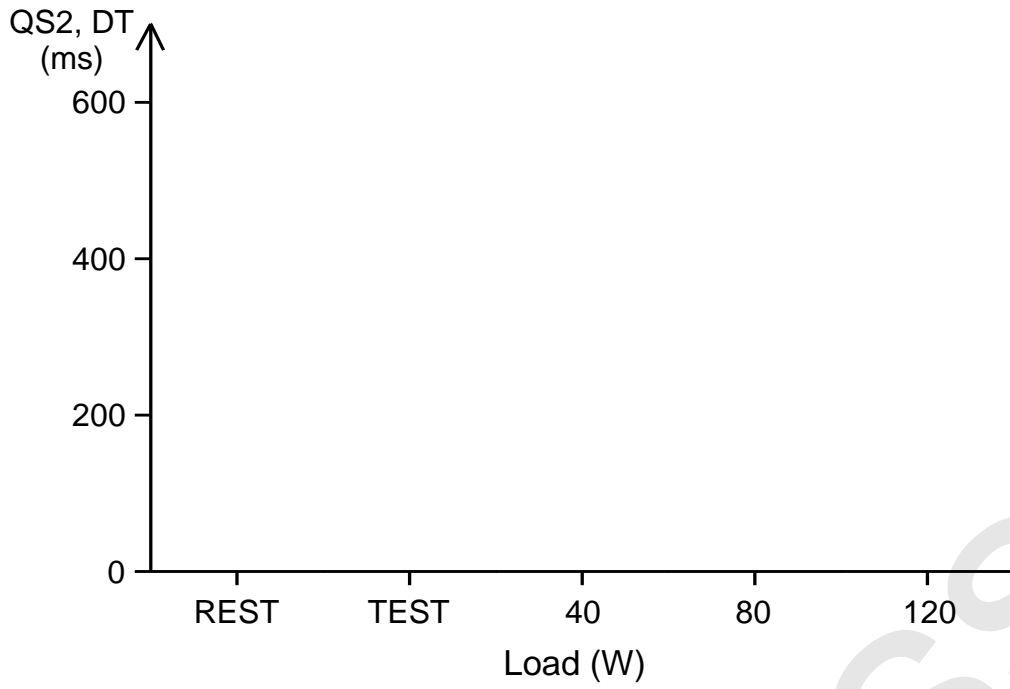
		Intensity of load: 40 W				Values during restitution					
Abbreviations	Parameters	0 min	1 min	2 min	3 min	1 min	2 min	4 min	6 min	8 min	10 min
P _S	Systolic blood pressure (mmHg)										
P _D	Diastolic blood pressure (mmHg)										
O ₂ Sat	Oxygen saturation (%)										
MABP	Mean arterial blood pressure (mmHg)										
HR	Heart rate (min ⁻¹)										
SV	Stroke volume (mL)										
CO	Cardiac output (L/min)										
SVR	Syst. vasc. resist. (dyn*sec*cm ⁻⁵)										
S	Ejection rate (ohm/sec)										
VET	Ventricular ejection time (ms)										
PEP	Preejection period (ms)										
QS2	Electromechanical syst. time (ms)										
DT	Diastolic period (ms)										

		Intensity of load: 80 W				Values during restitution					
Abbreviations	Parameters	0 min	1 min	2 min	3 min	1 min	2 min	4 min	6 min	8 min	10 min
P _S	Systolic blood pressure (mmHg)										
P _D	Diastolic blood pressure (mmHg)										
O ₂ Sat	Oxygen saturation (%)										
MABP	Mean arterial blood pressure (mmHg)										
HR	Heart rate (min ⁻¹)										
SV	Stroke volume (mL)										
CO	Cardiac output (L/min)										
SVR	Syst. vasc. resist. (dyn*sec*cm ⁻⁵)										
S	Ejection rate (ohm/sec)										
VET	Ventricular ejection time (ms)										
PEP	Preejection period (ms)										
QS2	Electromechanical syst. time (ms)										
DT	Diastolic period (ms)										

		Intensity of load: 120 W				Values during restitution					
Abbreviations	Parameters	0 min	1 min	2 min	3 min	1 min	2 min	4 min	6 min	8 min	10 min
P _S	Systolic blood pressure (mmHg)										
P _D	Diastolic blood pressure (mmHg)										
O ₂ Sat	Oxygen saturation (%)										
MABP	Mean arterial blood pressure (mmHg)										
HR	Heart rate (min ⁻¹)										
SV	Stroke volume (mL)										
CO	Cardiac output (L/min)										
SVR	Syst. vasc. resist. (dyn*sec*cm ⁻⁵)										
S	Ejection rate (ohm/sec)										
VET	Ventricular ejection time (ms)										
PEP	Preejection period (ms)										
QS2	Electromechanical syst. time (ms)										
DT	Diastolic period (ms)										

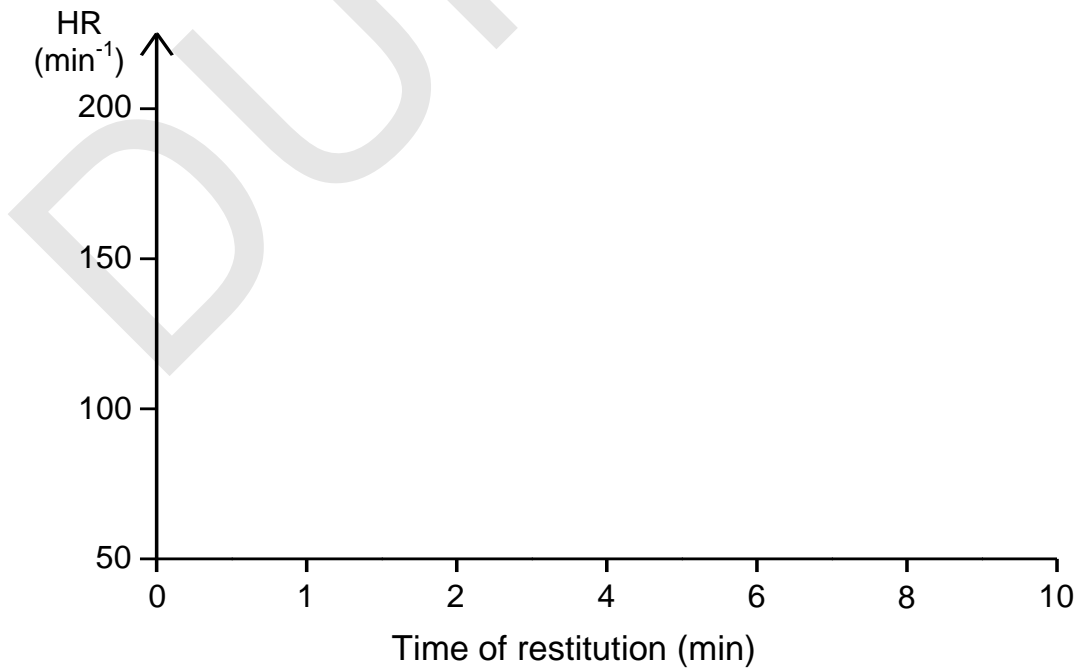
4.3. Plot the systolic and diastolic arterial blood pressure values (Ps and Pd), heart rate (HR), cardiac output (CO), stroke volume (SV), electromechanical systole time (QS2) and diastolic period (DT) **as the function of the intensity of the physical exercise**, using the values obtained at rest (REST), at the end of the lightest physical load (TEST) as well as at the end of the studied physical loads (40, 80, and 120 W).

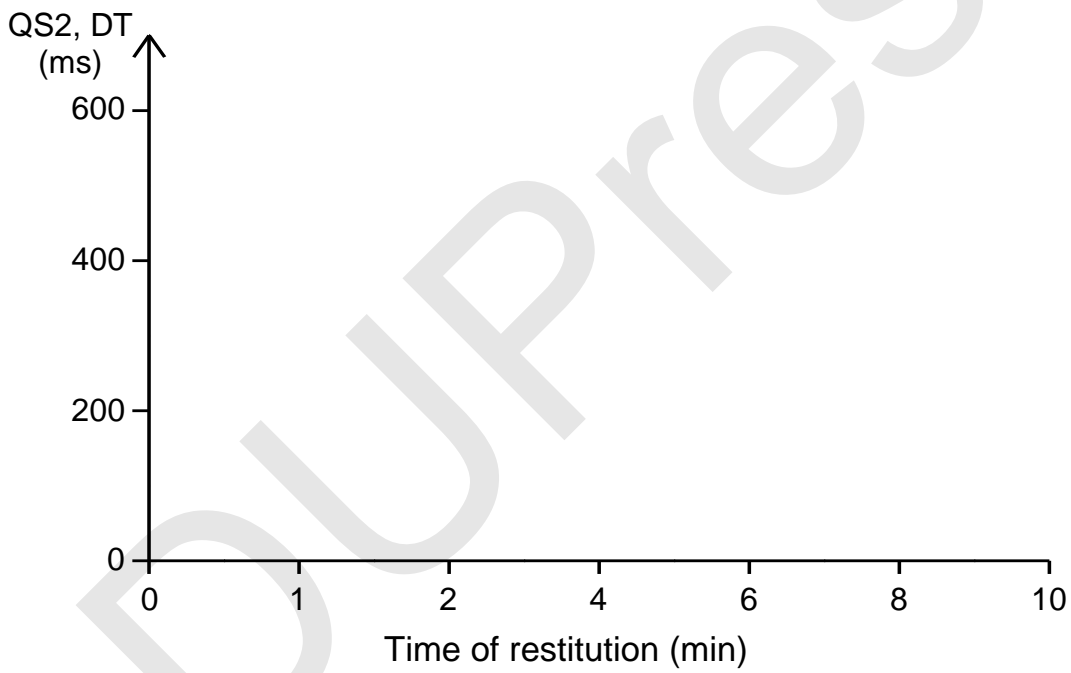
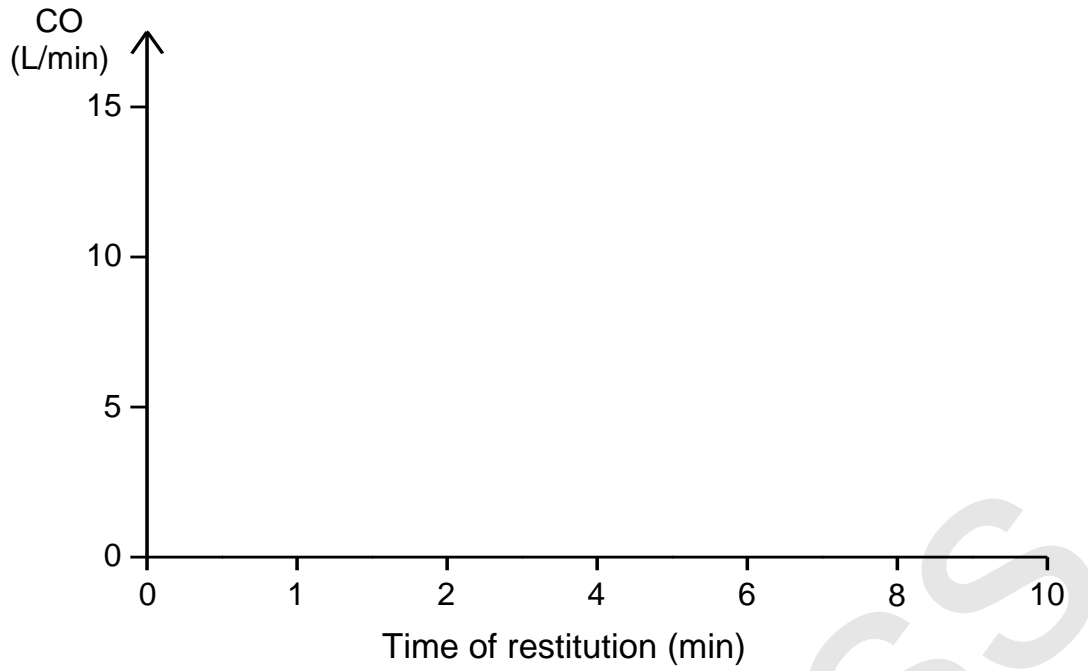




Summarize the results obtained during and after the physical loads and give a short explanation of your findings.

4.4. Plot the parameters **as the function of time during the restitution process**. Use the same graph to plot the restitution values after each load; different symbols or colours should distinguish between the individual loads.





Summarize the results (correlation between the physical exercise and the time course of restitution), and give a short explanation of your findings.

A representative ICG record with 4 curves:

DUPress

The student was present:

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date

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signature of lab teacher or helper

The lab is completed:

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date

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signature of lab teacher

TOPIC SHEET N° 5

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.

5.1. 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:

5.2. Examination of the 2nd cranial nerve

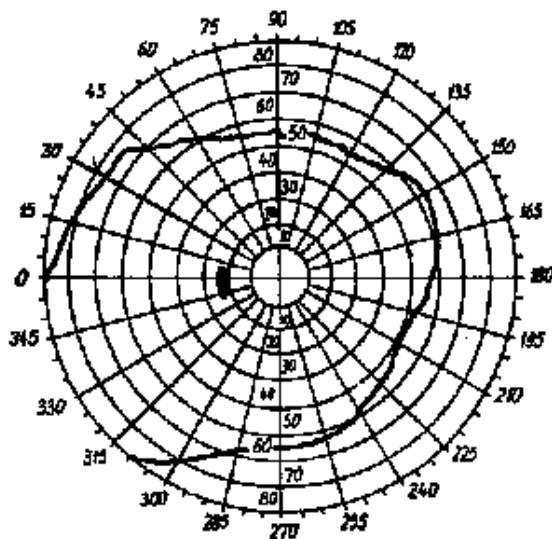
Anamnestic data (correction lens, scotoma or any visual disturbance):

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

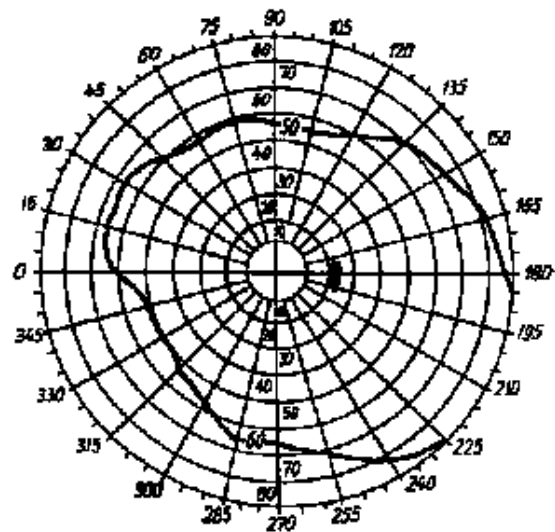
Visus of the left eye:

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.

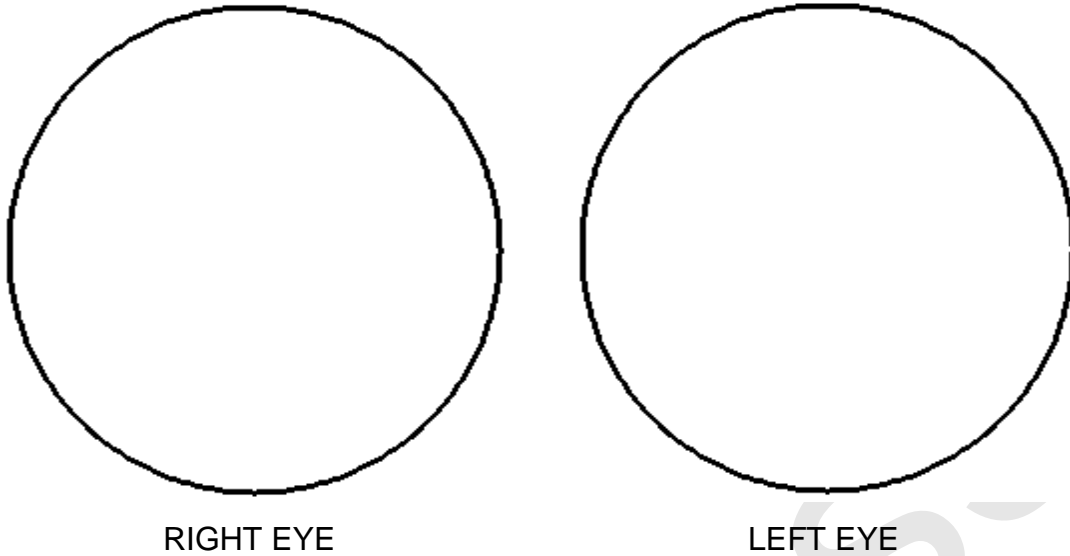


RIGHT EYE



LEFT EYE

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



Epicrisis:

5.3. 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:

5.4. Examination of the 5th cranial nerve

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

Anamnestic data:

Epicrisis:

5.5. Examination of the 7th cranial nerve

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

Anamnestic data:

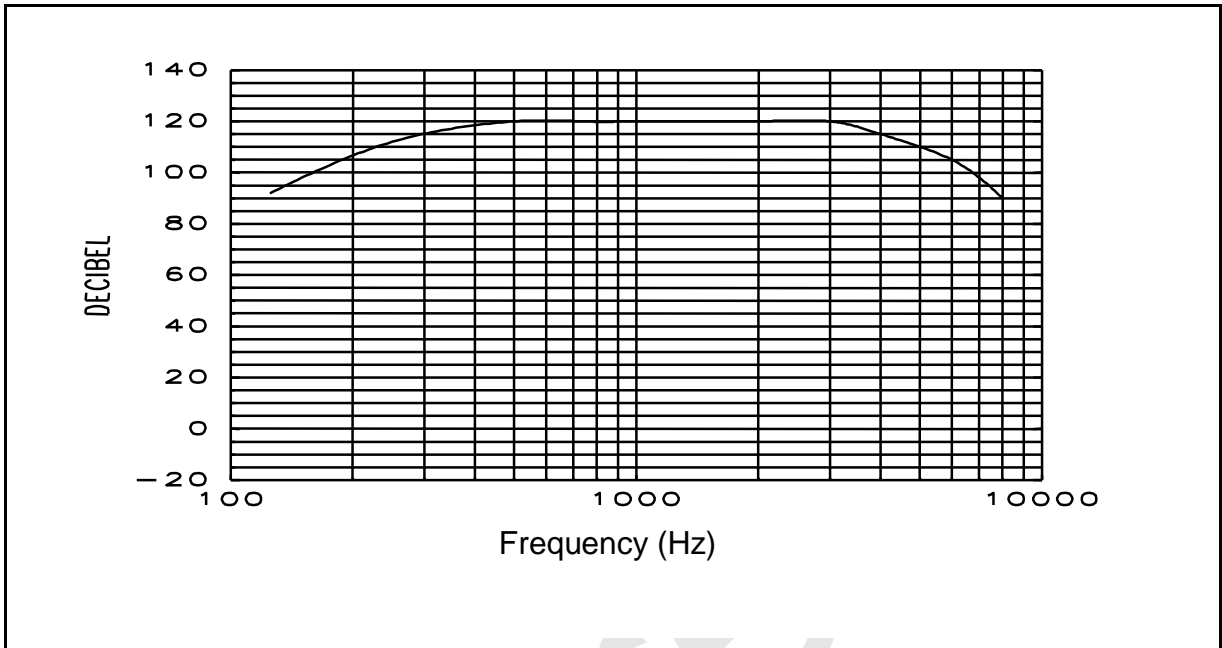
Epicrisis:

5.6. Examination of the 8th cranial nerve

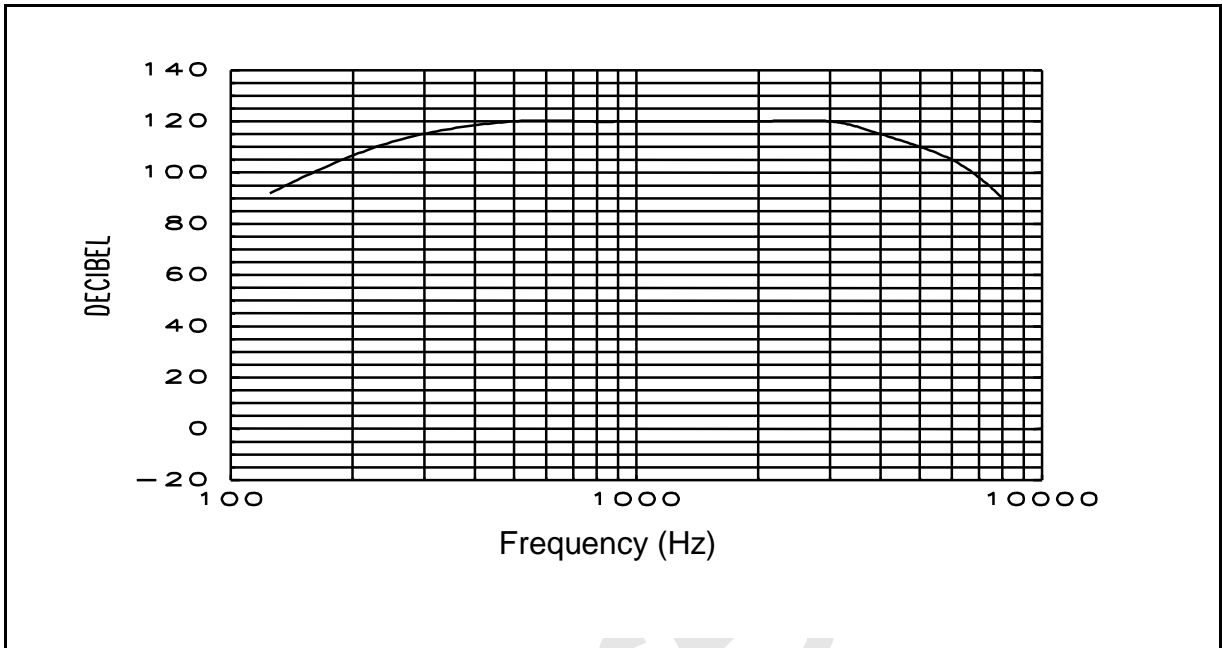
Examine the motor and sensory functions of the 8th cranial nerve.

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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date

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signature of lab teacher or helper

The lab is completed:

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date

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signature of lab teacher

TOPIC SHEET N° 6

EXAMINATION OF SOMATOSENSORY AND MOTORIC SYSTEMS

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

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

Anamnestic data:

Epicrisis:

6.2. Examination of the 11th cranial nerve

Examine the motor functions of the cranial nerve listed above.

Anamnestic data:

Epicrisis:

6.3. Examination of the 12th cranial nerve

Examine the motor functions of the cranial nerve listed above.

Anamnestic data:

Epicrisis:

6.4. Examine the motor functions of your colleague.

Anamnestic data:

Epicrisis:

6.5. Examine the sensory functions of your colleague.

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

Anamnestic data:

Epicrisis:

6.6. 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).

+ > > > > > -

6.7. 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:

6.8. 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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date

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signature of lab teacher or helper

The lab is completed:

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date

.....
signature of lab teacher

TOPIC SHEET N° 7A.

EXAMINATION OF THE BLOOD I.

7.1.1. Study and compare the transport properties for thiocarbamide, glucose, glycerol and NH_4Cl . Mix blood samples one by one with each solution, and record the changes in extinction as the function of time. Read the extinctions right after mixing the solutions (0 min) as well as 1, 4 and 5 min afterwards. Compare the changes in the extinction occurring during the 1st (E_0-E_1) and the 5th (E_4-E_5) min, too. Changes should be recorded for 6 minutes using 50 cm/h paper velocity; in the case of thiocarbamide the recording should last until complete hemolysis is achieved, using paper velocity of 5 cm/min.

	VALUE OF EXTINCTION					
	0 min	1 min	4 min	5 min	E_0-E_1	E_4-E_5
thiocarbamide						
glycerol						
NH_4Cl						
glucose						

RECORD:

7.1.2. Determine the osmotic resistance of control (untreated) and thiocarbamide-treated blood samples. Hemolysis is monitored as a reduction of extinction. Record the changes in extinction, and complete the table below by reading the extinctions. Please, enclose the original records as well.

Concentration of thiocarbamide applied: mmol/L.

(To be specified by the tutor.)

	CONTROL BLOOD	THIOCARBAMIDE-TREATED BLOOD
0.90 %		
0.80 %		
0.70 %		
0.65 %		
0.60 %		
0.55 %		
0.50 %		
0.45 %		
0.40 %		
0.30 %		
0.20 %		

RECORDS:

TOPIC SHEET N° 7B.

EXAMINATION OF THE BLOOD II.

Summarize the characteristic parameters of two blood samples and fill in the table below.

Carry out the experiments using samples N° and

Parameter	Value	Value
Red Blood Cell count (T/L)		
Hemoglobin concentration (g/L)		
Mean Corpuscular Hemoglobin (pg)		
Red Cell Index		
Hematocrit (%)		
Mean Corpuscular Volume (fL)		

Evaluation:

The student was present:

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date

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signature of lab teacher or helper

The lab is completed:

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date

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signature of lab teacher

TOPIC SHEET N° 8

COMPUTER AIDED ACQUISITION AND PROCESSING OF BIOLOGICAL SIGNALS

8.1. 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)	1 st point (V)	2 nd point (V)	3 rd point (V)	4 th point (V)	5 th point (V)	Mean (V)
0						
1						
2						
5						
10						
20						
30						
50						

Plot the force as the function of the measured voltage.



Close temporarily 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.

8.2. 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; $mN \cdot s$)								
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 1 st marker	Between the 1 st and 2 nd markers	After the 2 nd marker
Maximal rate of rise (s^{-1})			
Area under the curve (integral; $mN \cdot s$)			
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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TOPIC SHEET N° 9

STUDYING THE FUNCTION OF PERIPHERAL NERVES AND THE INNERVATED MUSCLES

9.1. 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	1 st	2 nd	3 rd	4 th	5 th
Conduction velocity (m/s)					
Relative proportion (% of 1 st)					

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

9.2. 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.

<p>receptor potential</p> 	<p>tonic receptor</p> 	
<p>phasic/tonic receptor</p> 	<p>phasic receptor</p> 	

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?

9.3. 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

9.4. 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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TOPIC SHEET N° 10**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.

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

RECORD:

File name:

Gain:

10.2. 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)			

10.3. 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:

DUPRESS

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+}	$\text{Mg}^{2+} + \text{Ca}^{2+}$	WASHOUT
F (mN)				
TTP (s)				
HRT (s)				

10.4. 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:

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signature of lab teacher

TOPIC SHEET N° 11**EFFECTS OF NEUROTRANSMITTERS AND HORMONES ON THE UTERINAL SMOOTH MUSCLE FUNCTION**

Myometrium stripes isolated from adult female rats are used to study the function and pharmacological characteristics 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 (10-15 min) on smooth muscle contraction. In order to avoid disturbances in drug-actions, a minimum of 10 to 15 min of 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.

11.1. Effects of neurotransmitters and their antagonists

11.1.1. Record the contractile pattern of the uterine stripe in Tyrode solution then add 50 μ L of **epinephrine** solution (Tonogen ampoule, containing 1 mg/mL epinephrine). After recording the effect of epinephrine, add 50 μ L of **pindolol** solution to the bath, and continue recording until the spontaneous mechanical activity returns. Finally, switch to normal Tyrode solution.

RECORD:

File name:

Gain:

11.1.2. After the recovery of the normal activity of the preparation, add 100 μL of **acetylcholine** solution (10 $\mu\text{g}/\text{mL}$). After recording the effect of acetylcholine, add 50 μL of **atropine** solution (Atropinum sulfuricum ampoule, containing 1 mg/mL atropine). Continue recording until the effect of acetylcholine attenuates, and return to drug-free 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 contraction (TTP)
- half-relaxation time (HRT)
- slope of rising phase of contraction (Slope)
- Integral

	CONTROL	ACETYLCHOLINE	ACETYLCHOLINE + ATROPINE
CL (s)			
F (mN)			
TTP (s)			
HRT (s)			
Slope (mN/s)			
Integral (mN*s)			

11.1.3. After a washing period of 10-15 min, apply 40 μL of **phenylephrine** solution (10 mM stock solution of phenylephrine). Record the effect of phenylephrine on the contractile pattern then add 50 μL of **phentolamine** solution (Regitin ampoule, containing 10 mg/mL phentolamine) 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.

RECORD:

File name:

Gain:

DUPress

Measure the following parameters of the recorded smooth muscle contractions:

	CONTROL	PHENYLEPHRINE	PHENYLEPHRINE + PHENTOLAMINE
CL (s)			
F (mN)			

11.2. Effects of uterotonic drugs

Record the spontaneous activity for 2-3 minutes.

11.2.1. 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).

RECORD:

File name:

Gain:

DUPress

Measure the duration of smooth muscle contractions elicited by the first and second histamine treatments.

Duration of contraction:

- in the control phase: s
- during the first histamine treatment: s
- during the second histamine treatment: s

11.2.2. Add 100 μL **oxytocin** (Oxytocin ampoule, 5 IU/mL) to the tissue bath of myometrium showing normal mechanical activity. After recording the effect of oxytocin for 10-15 min, add 20 μL of **papaverine** solution (Papaverine hydrochloricum ampoule, containing 40 mg/mL papaverine) to the bath, and continuously record the effect of papaverine on the uterinal contraction. Finally, return to drug-free Tyrode solution.

RECORD:

File name:

Gain:

Measure the following parameters of the smooth muscle contraction:

	CONTROL	OXYTOCIN TREATMENT
CL (s)		
F (mN)		
TTP (s)		
HRT (s)		
Slope (mN/s)		
Integral (mN*s)		

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TOPIC SHEET N° 12

SIMULATION OF THE ACTION POTENTIAL IN THE SQUID AXON

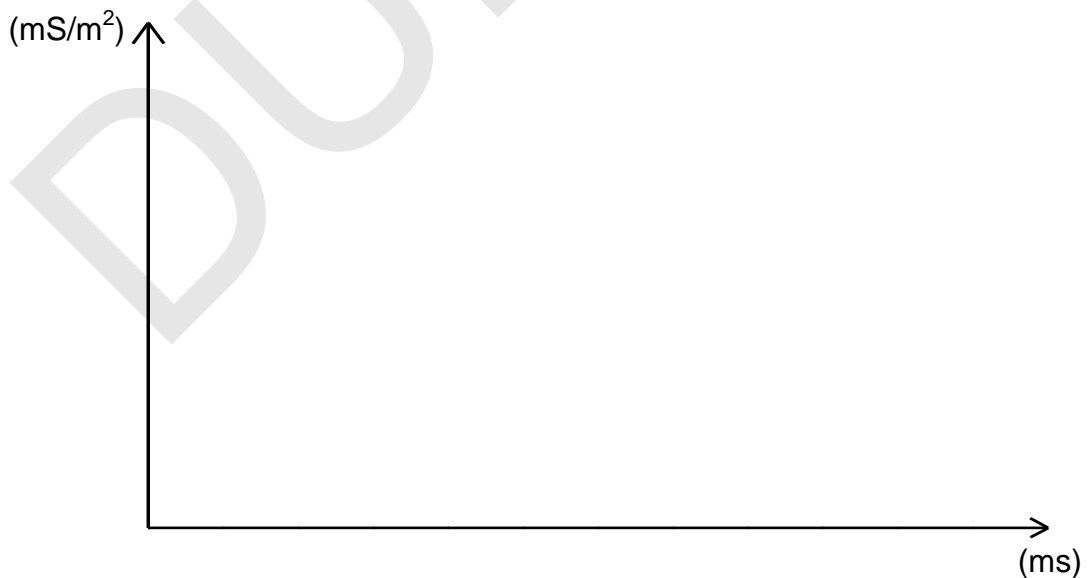
12.1. Activation threshold of the cell membrane

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:.....($\mu\text{A}/\text{cm}^2$)

12.2. 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.

	1 st pulse	2 nd pulse
amplitude:
delay:	

12.3. 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!

	1 st pulse	2 nd 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!

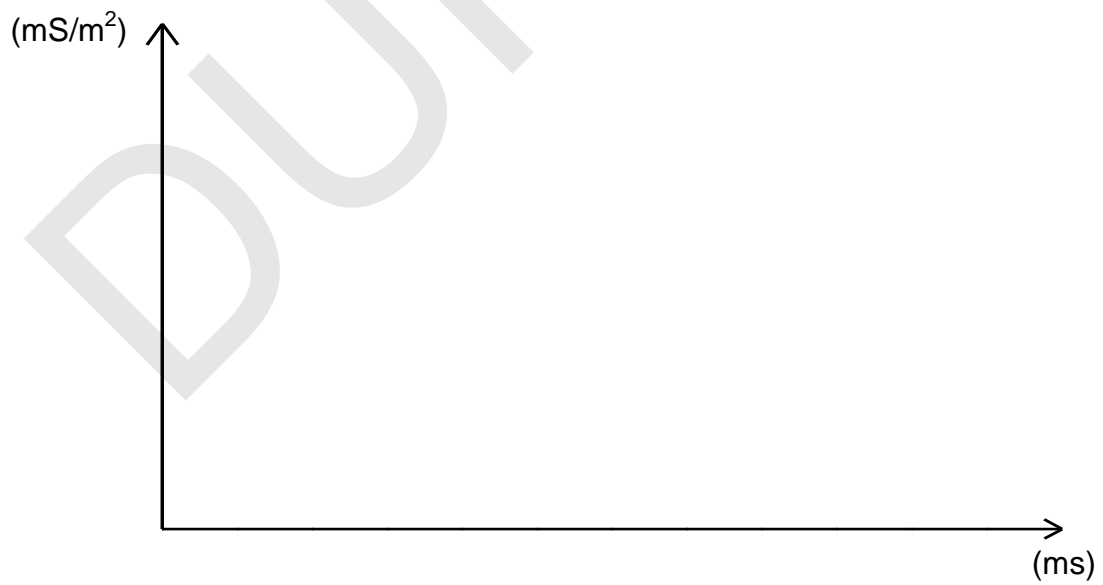
	1 st pulse	2 nd pulse
amplitude:
duration:
shortest required time:	

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

potential changes:



conductance changes:



12.5. 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 μA/cm²) 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!

12.6. 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	



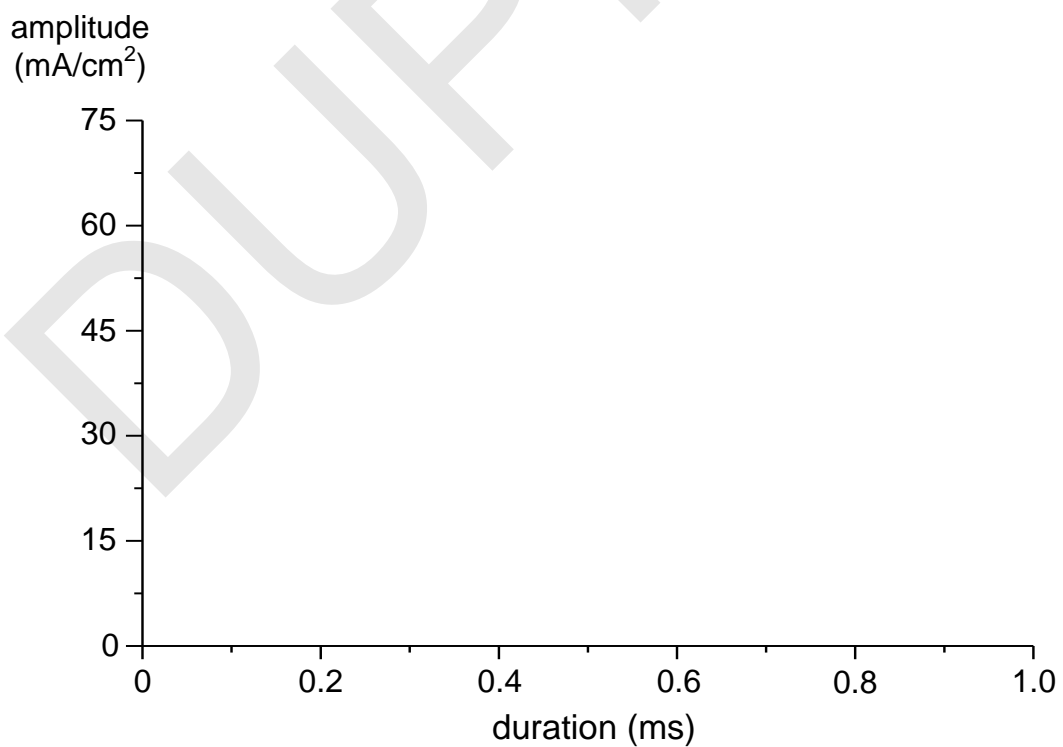
Summarize the major differences observed!

12.7. 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 msmA/cm ²
0.2 msmA/cm ²
0.3 msmA/cm ²
0.4 msmA/cm ²
0.5 msmA/cm ²
0.6 msmA/cm ²
0.7 msmA/cm ²
0.8 msmA/cm ²
0.9 msmA/cm ²
1.0 msmA/cm ²

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



12.8. Effects of different drugs on the action potential morphology

12.8.1. 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 $\mu\text{A}/\text{cm}^2$ 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!

12.8.2. 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

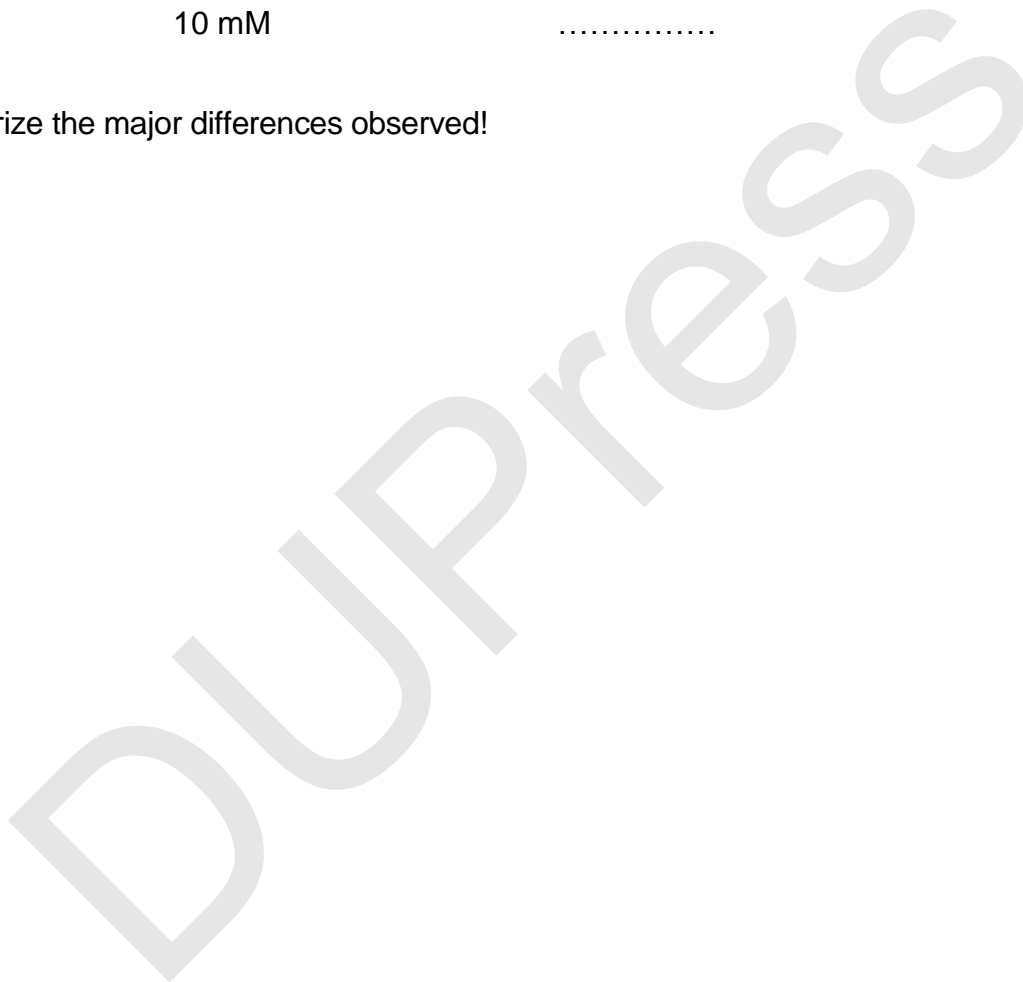


Summarize the major differences observed!

12.8.3. 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!



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TOPIC SHEET N° 13

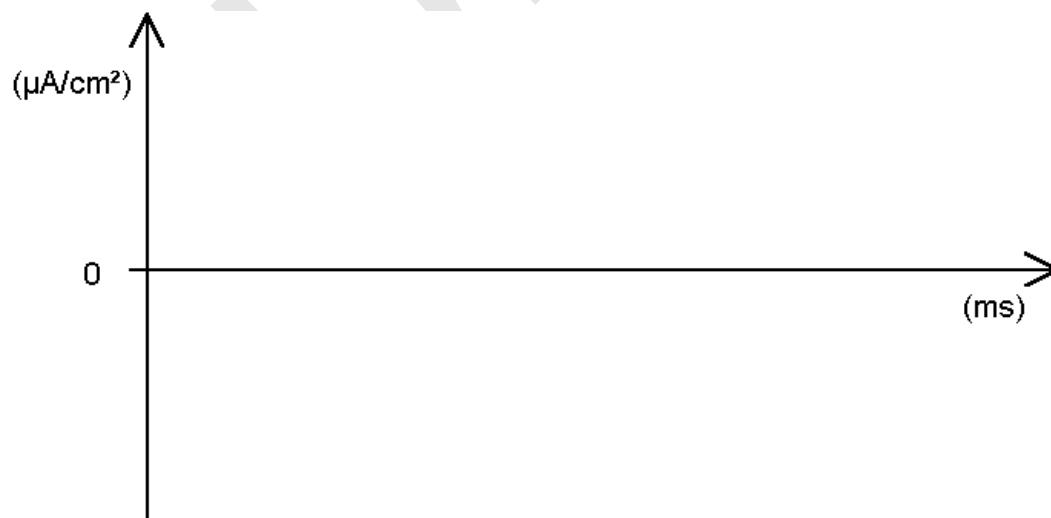
SIMULATION OF THE IONIC CURRENTS IN THE SQUID AXON

13.1. Depict the specific membrane conductance changes and total ionic currents when the membrane potential is set to -10 mV for 15 ms by selecting the 4th point of the MENU! Use 25 ms long simulation duration in the display menu!

conductance changes:



ionic currents:

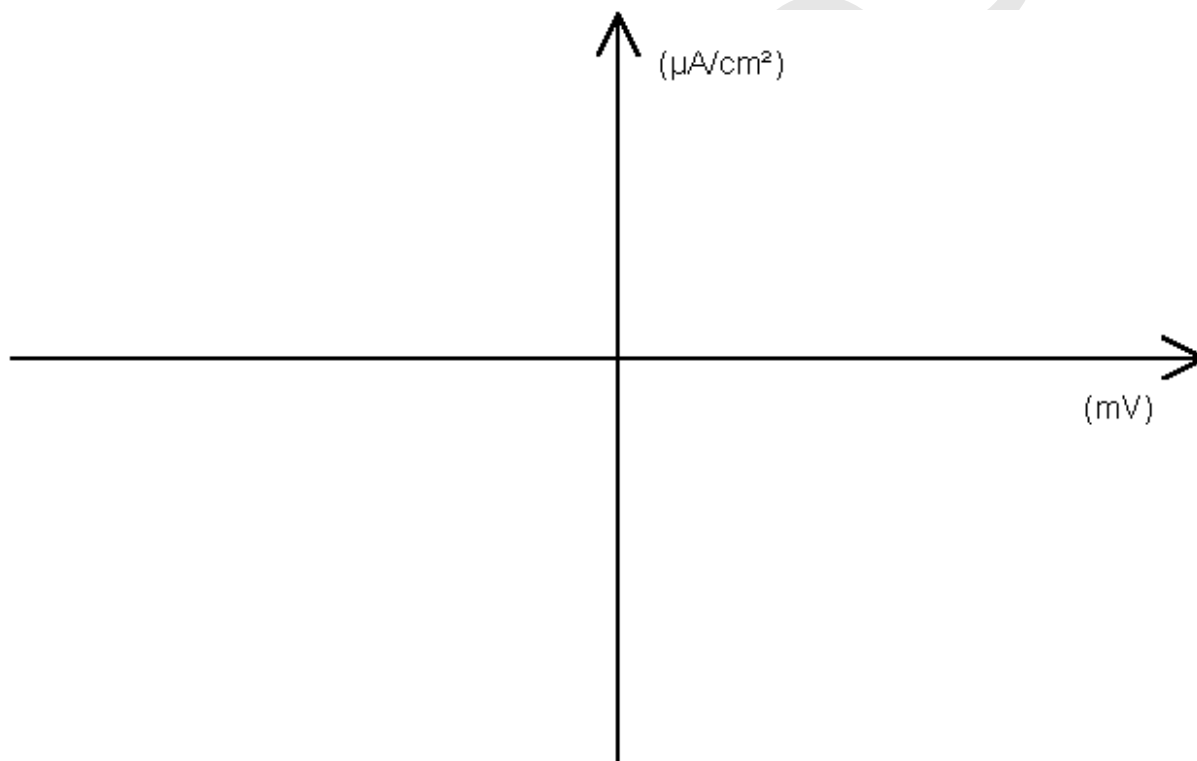


What is the difference between the kinetics of two channel conductances and currents?
Why?

13.2. Current-voltage relationship of Na⁺ current under different conditions

Determine the current-voltage relationship and the equilibrium potential for Na⁺ (E_{Na}) between -50 and +90 mV! Repeat the measurement in the presence of 1 nM saxitoxin (a Na⁺ channel blocker) and when the Na⁺ gradient is increased.

membrane potential (mV)		maximal current (mA/cm ²)		
		control	1 nM saxitoxin	increased Na ⁺
1.	-50
2.	-30
3.	-10
4.	0
5.	+10
6.	+40
7.	+60
8.	+90

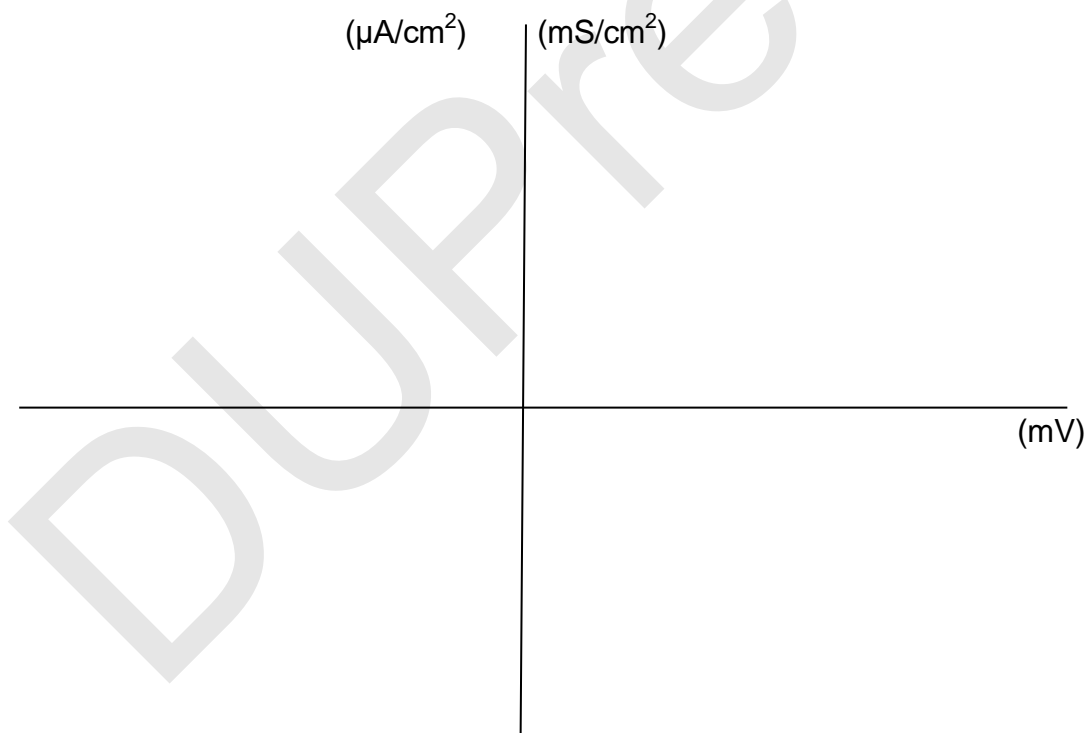


	control	1 nM saxitoxin	increased Na ⁺
Value of E _{Na} (mV).

13.3. Comparison of the voltage dependence of the channel conductance and ionic current

Plot the relationships between the voltage, peak of the sodium channel conductance and current between -50 and +90 mV (holding potential -100 mV, impulse duration 6 ms, time scale 10 ms).

membrane potential (mV)	max. current (mA/cm ²)	max. cond. (mS/cm ²)
1. -50
2. -30
3. -10
4. 0
5. +10
6. +40
7. +60
8. +90

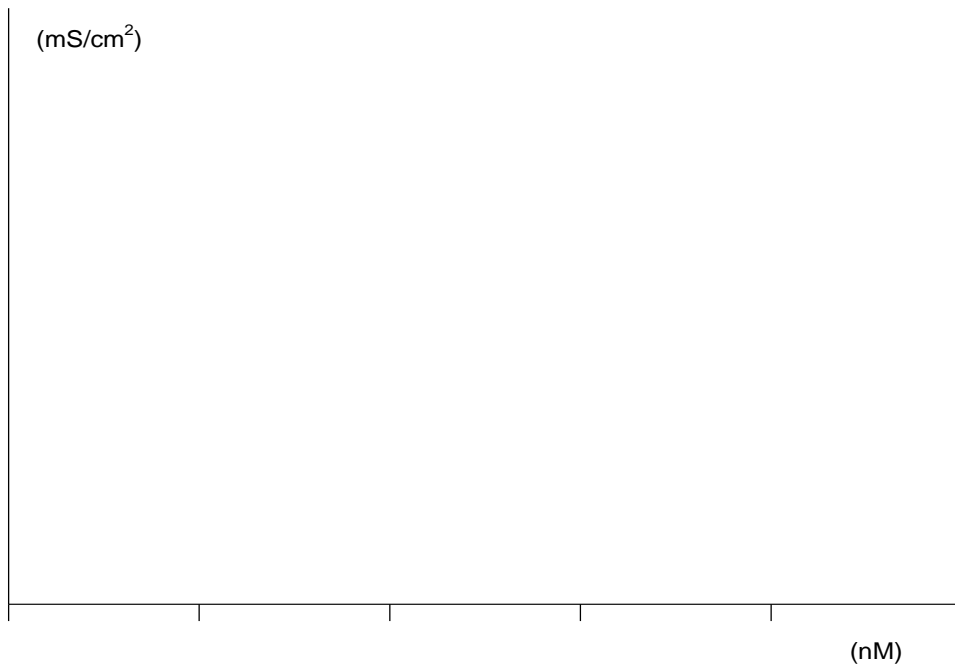


What is the difference between the voltage dependence of the sodium conductance and the sodium current? Why are they different?

13.4. The dose dependence of the saxitoxin action

Determine the dose-dependence curve of the saxitoxin (between 0.1 – 5 nM) on the sodium channel conductance.

STX (nM)	maximal Na ⁺ conductance (mS/cm ²)
1. 0
2. 0.1
3. 0.2
4. 0.5
5. 1
6. 2
7. 5



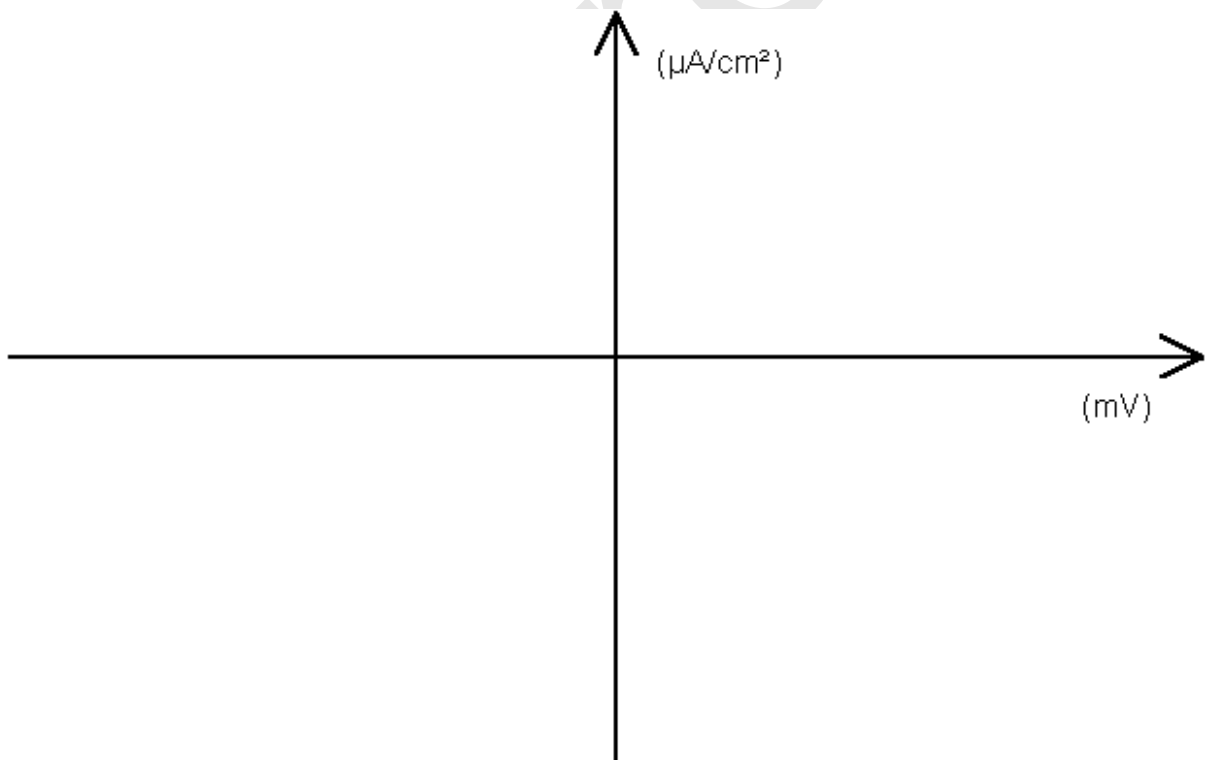
IC₅₀ of saxitoxin: nM

13.5. Current-voltage relationship of the K⁺ current under different conditions

Determine the current-voltage relationship of the K⁺ current between -50 and +90 mV!

Repeat the measurement in the presence of 1 mM TEA (a K⁺ channel blocker) and when the K⁺ gradient is increased.

membrane potential (mV)	maximal ionic current (mA/cm ²)		
	control	1 mM TEA	increased K ⁺
-50
-30
-10
0
10
30
50
70
90



13.6. Comparison of Na⁺ and K⁺ currents

Make a comparison between the Na⁺ and K⁺ currents under different membrane potentials (-20, 20 and 60 mV). Summarize the major differences observed! What is the explanation of the differences?

DUPress

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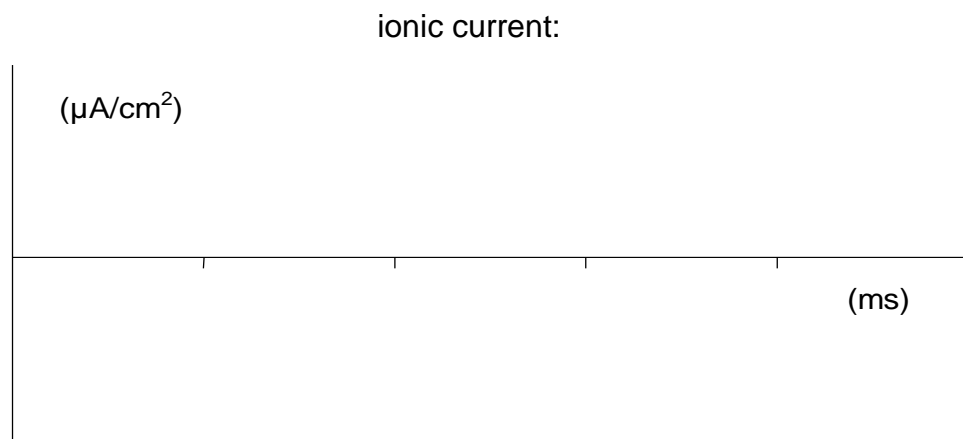
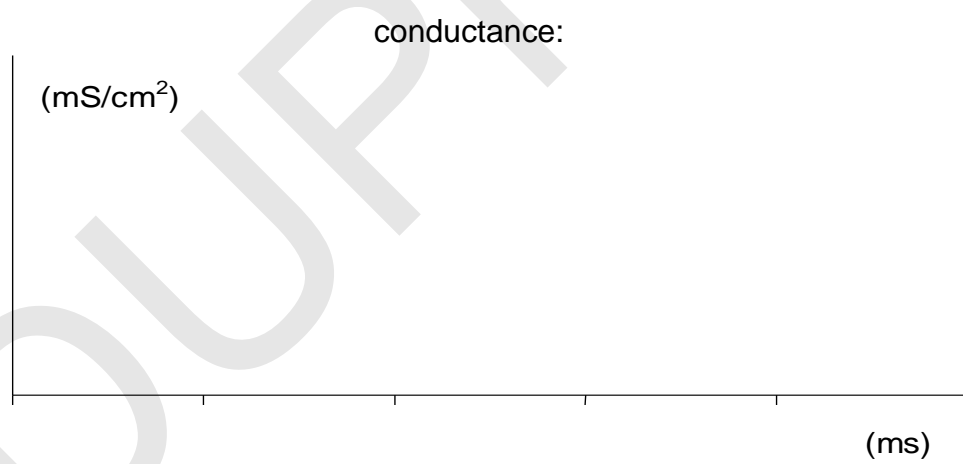
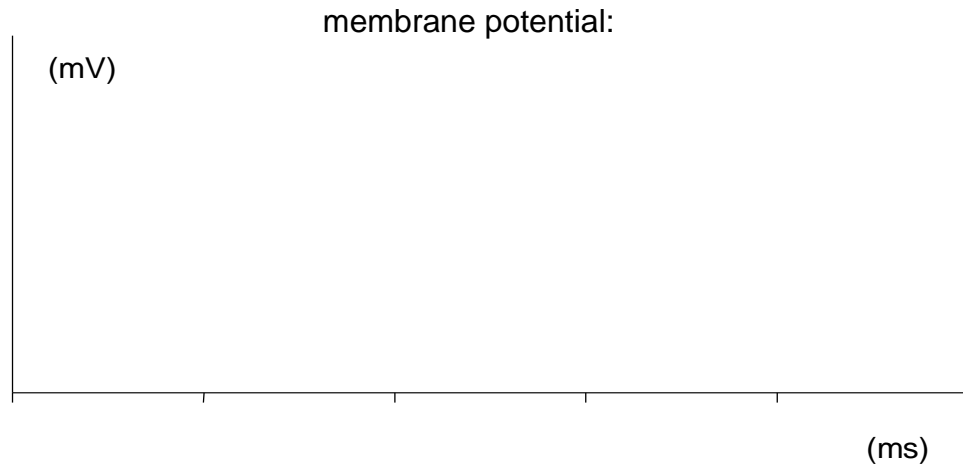
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TOPIC SHEET N° 14

COMPUTER SIMULATION OF CARDIAC ACTION POTENTIALS

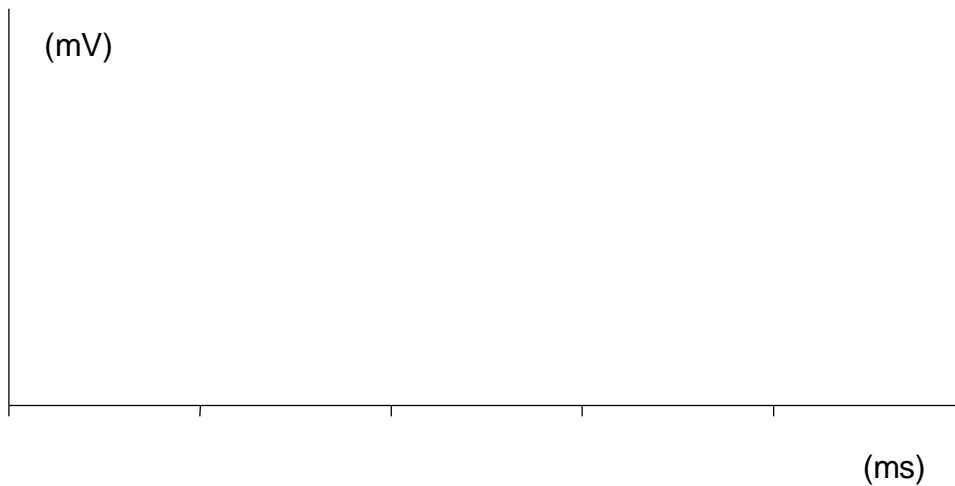
14.1. Demonstrations

14.1.1. By selecting the 1st point of DEMONSTRATIONS, plot a **fast action potential** together with the changes in ionic conductances and ionic currents.

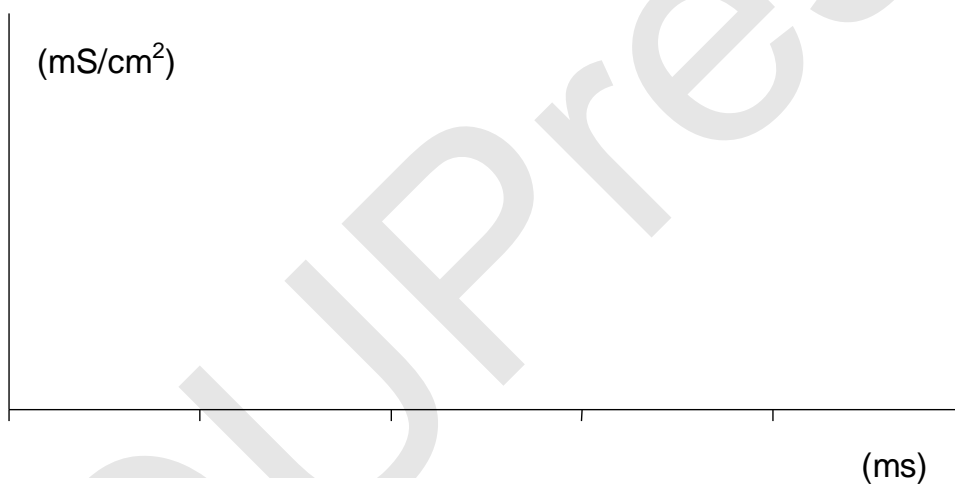


14.1.2. By selecting the 2nd point of DEMONSTRATIONS, plot a **slow action potential** together with the changes in ionic conductances and ionic currents.

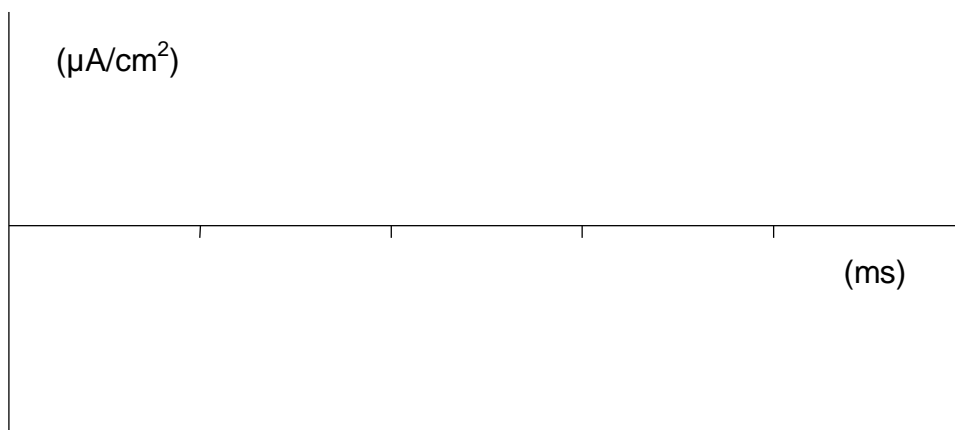
membrane potential:



conductance:



ionic current:



14.1.3. Describe the effects of changing the **Na⁺ equilibrium potential** (E_{Na}) on the fast action potential and on the Na⁺ current.

	E_{Na} decreased	E_{Na} normal	E_{Na} increased
Action potential			
amplitude (mV):
rate of rise: (decreased or increased; Relative to the normal)
Na ⁺ current			
amplitude ($\mu A/cm^2$):
rate of rise: (decreased or increased; Relative to the normal)

14.1.4. Describe the effects of changing the **Ca²⁺ equilibrium potential** (E_{Ca}) on the parameters of the fast action potential and on the Ca²⁺ current.

	E_{Ca} decreased	E_{Ca} normal	E_{Ca} increased
Duration of plateau phase (ms) ($V_m > -40$ mV):
Ca ²⁺ current			
amplitude ($\mu A/cm^2$):
duration (ms):

14.1.5. Describe the effects of changing the **K⁺ equilibrium potential (E_K)** on the parameters of the fast action potential and the K⁺ current.

	E _K decreased	E _K normal	E _K increased
Duration of plateau phase (ms):
K ⁺ current amplitude (μA/cm ²):
duration (ms):

14.1.6. By selecting the 6th point of DEMONSTRATIONS, study a control series of action potentials and then the effects of changing the amplitude of the **steady Na⁺ current** on the repetitive activity. Denote the main characteristics as mentioned above.

	Na ⁺ current		
	2.3 (μA/cm ²)(μA/cm ²)(μA/cm ²)
rate of rise of the prepotential: (decreased or increased; Relative to the normal)	
frequency of the action potentials (Hz):

14.1.7. Study the changes in the **pacemaker activity** induced by sympathetic or parasympathetic stimulation. Give a brief description of the observed effects.

Adrenergic effects:

Cholinergic effects:

14.1.8. Study the changes in the **myocardial action potential** induced by adrenergic or cholinergic stimulation. Give a brief description of the observed effects.

Adrenergic effects:

Cholinergic effects:

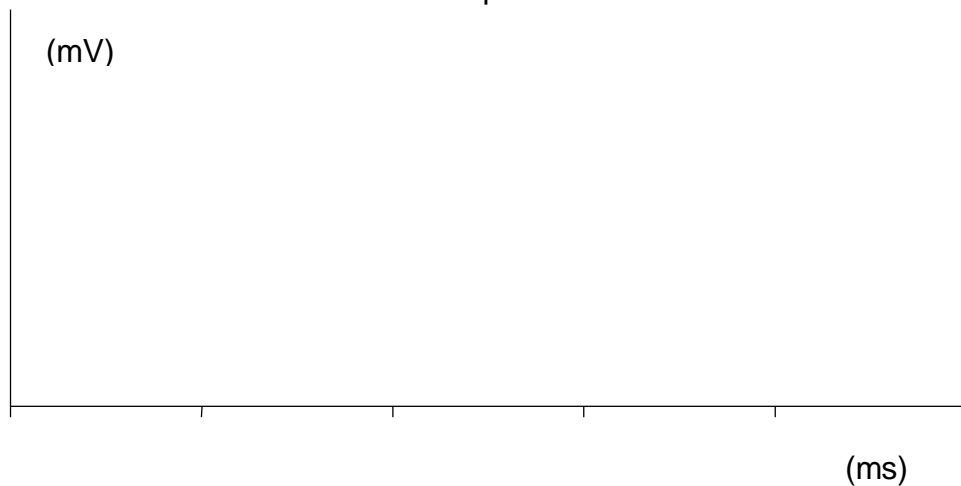
14.2. Simulations with user-defined data

14.2.1. By selecting the 2nd point of MENU, determine the threshold stimulus of a myocardial cell.

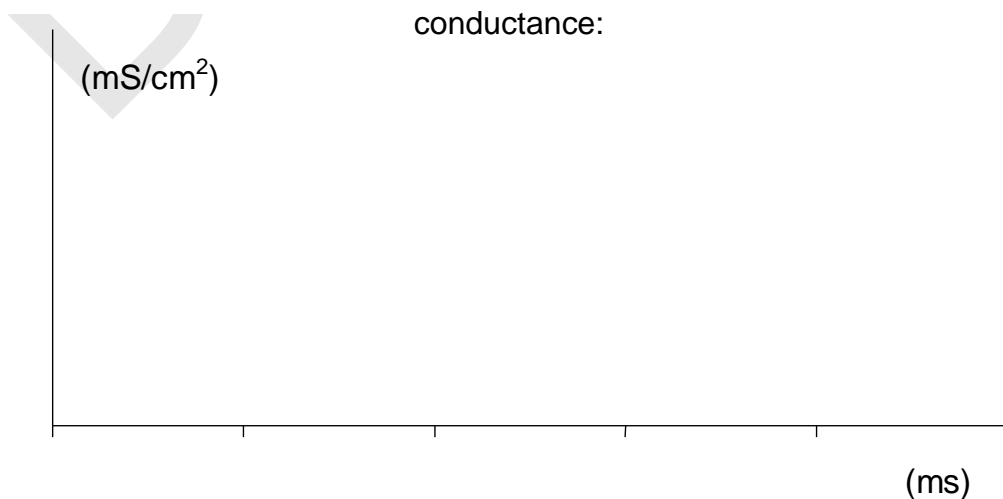
Threshold stimulus: mV

Draw the changes of the membrane potential and of the ionic conductances during the first 10 ms.

membrane potential:

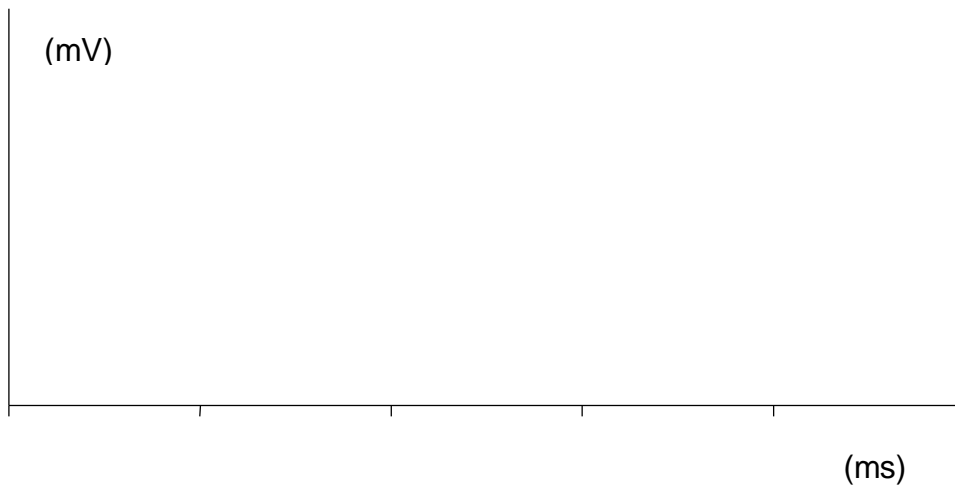


conductance:

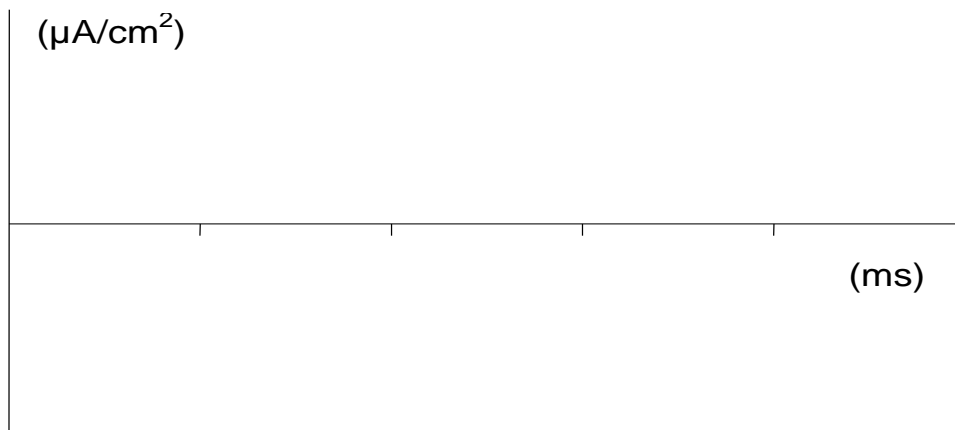


14.2.2. By selecting the 3rd point of MENU, repeat the experiment with the data used above. Together with the action potential draw the changes of the ionic currents.

membrane potential:



ionic current:

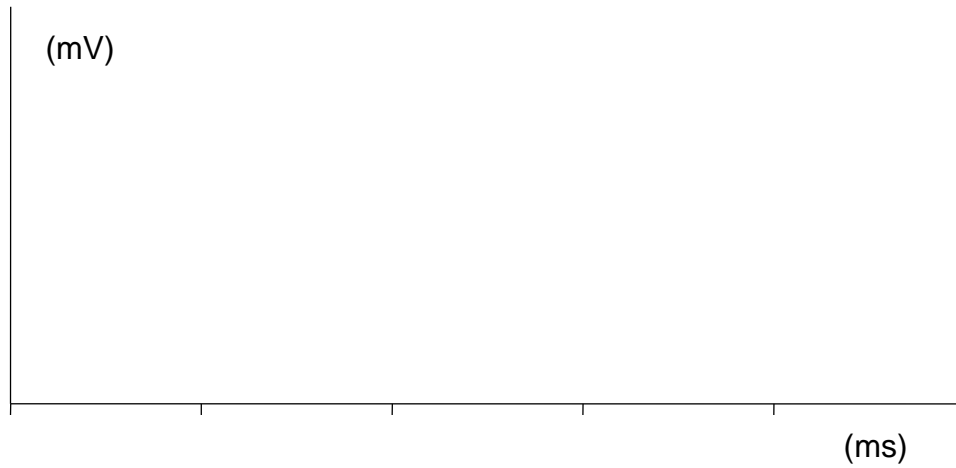


14.2.3. Find the most negative resting membrane potential value at which action potentials cannot be generated due to the **steady-state inactivation** of the Na⁺ channels.

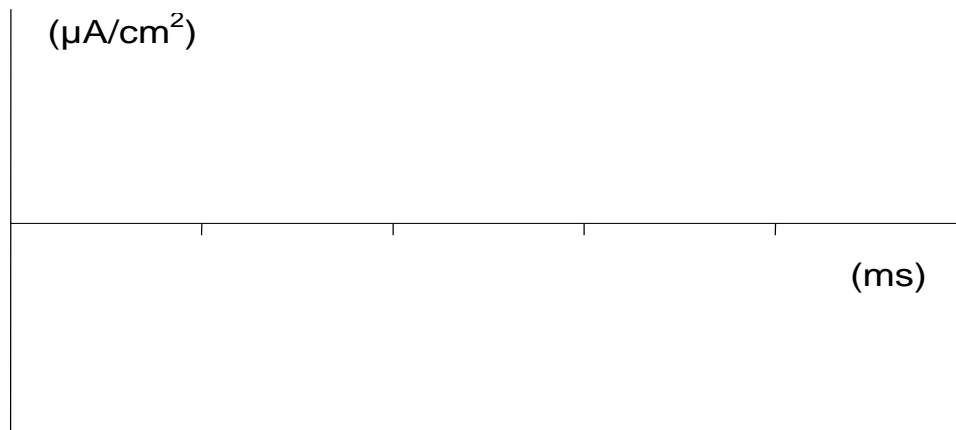
resting membrane potential: mV

14.2.4. Demonstrate the effects of changing the resting membrane potential to 0 mV on the rate of rise of the action potential, on the overshoot and on the Na⁺ current during the first 10 ms. Compare the results obtained at -85 mV with those recorded at two other resting membrane potential values.

action potential

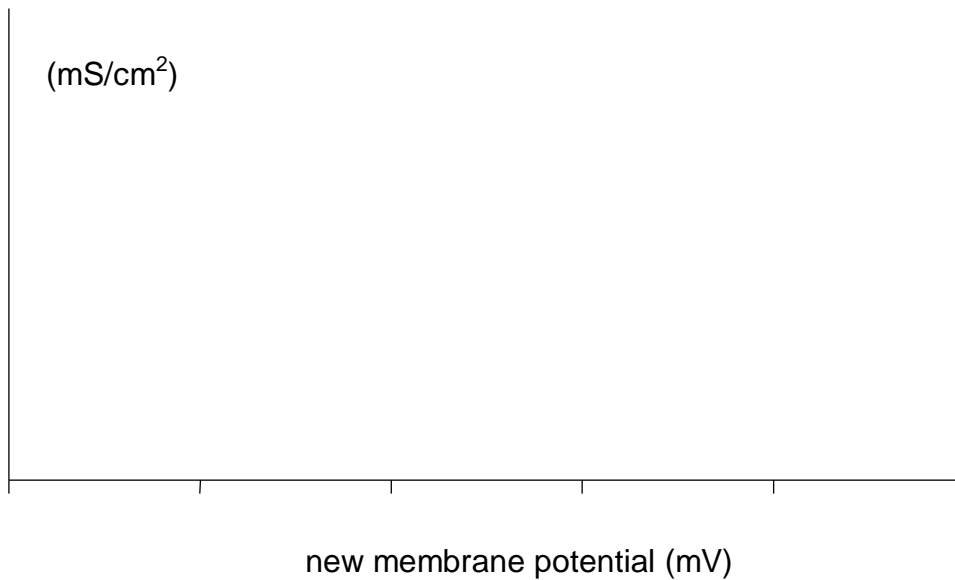


Na⁺ current

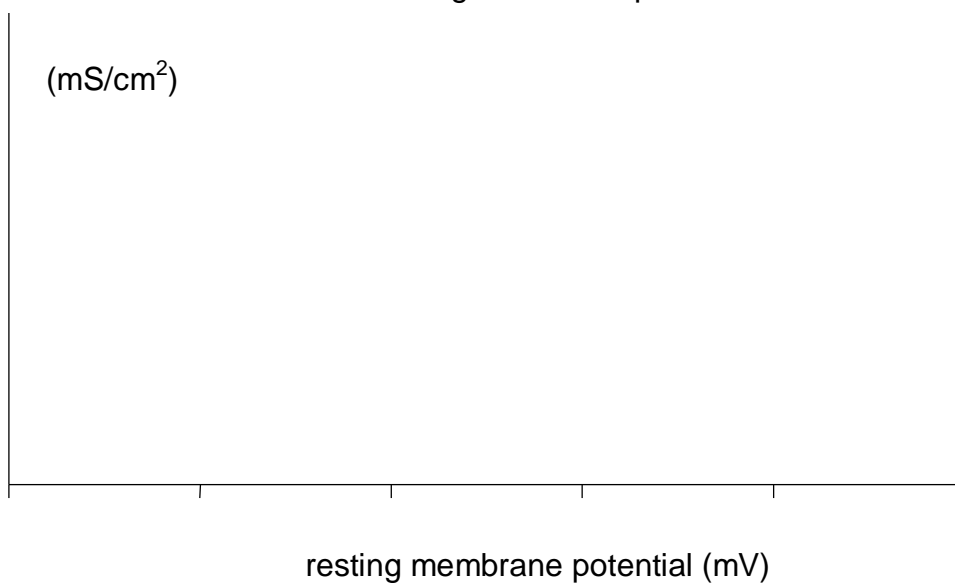


Interpret your observations.

14.2.5. While keeping the resting membrane potential set to -85 mV, vary the new membrane potential between -65 and -40 mV. Plot the peak of the Na⁺ conductance as the function of the new membrane potential. Use 0.2 mV increments for changing the new membrane potential in a ± 1 mV interval around the previously determined threshold potential.



14.2.6. While keeping the new membrane potential set to 0 mV, vary the resting membrane potential between -100 and -50 mV in 5 mV steps. Plot the peak of the Na⁺ conductance as the function of the resting membrane potential.



Interpret the obtained graphs.

The student was present:

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date

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signature of lab teacher or helper

The lab is completed:

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date

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signature of lab teacher

TOPIC SHEET N° 15**SIMULATION OF THE CARDIAC CYCLE AND THE STARLING MECHANISM****15.1. EVENTS OF THE CARDIAC CYCLE****15.1.1. 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?

15.1.2. 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!

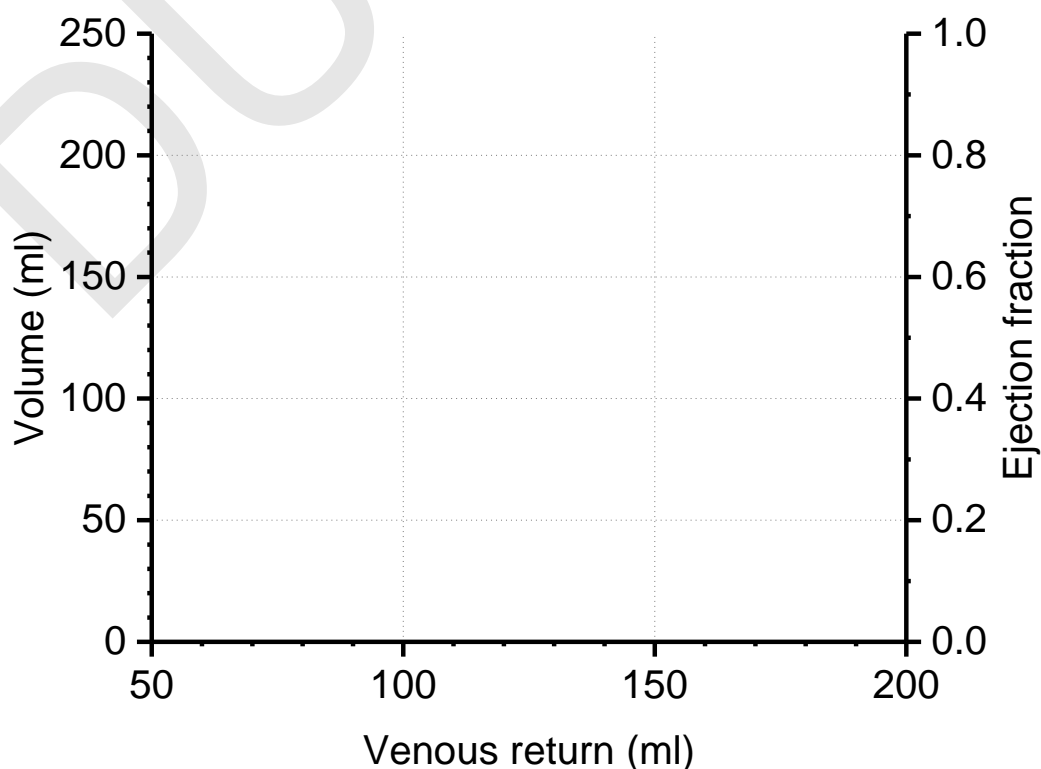
DUPress

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

15.2.1. 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*!

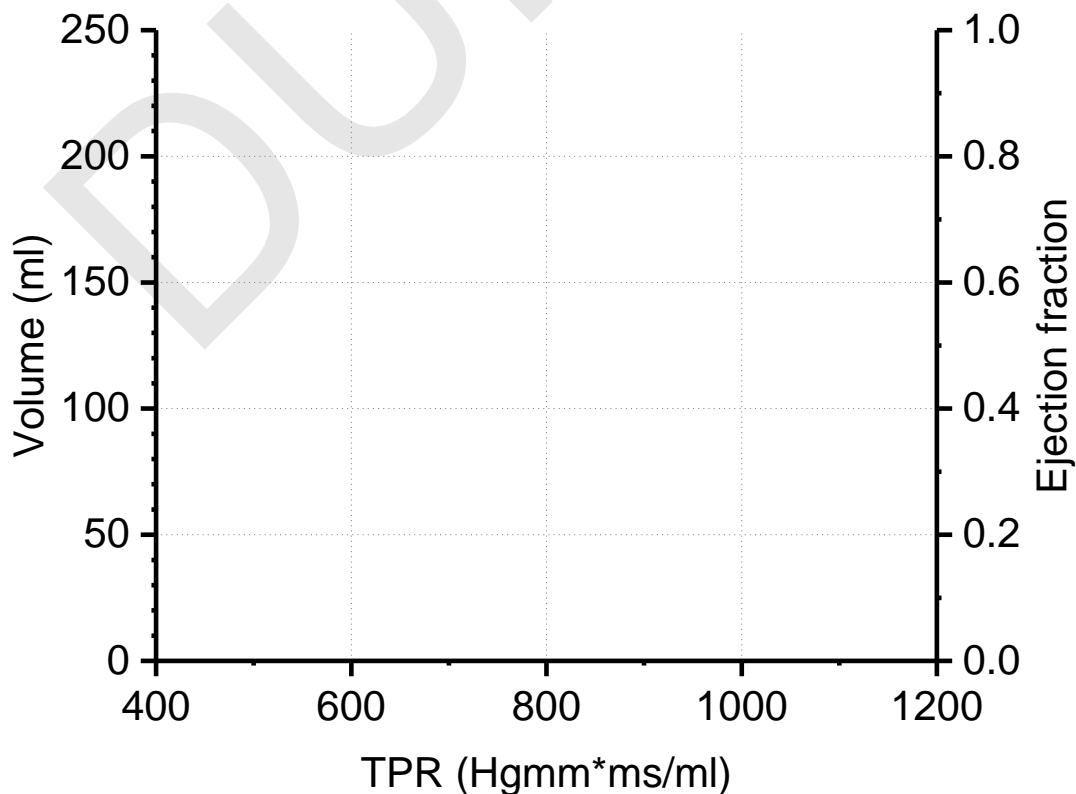
Venous return (ml)	End-diastolic volume (ml)	End-systolic volume (ml)	Stroke volume (ml)	Ejection fraction	Aortic pressure [sys/dia] (Hgmm)
60					/
80					/
100					/
120					/
140					/
160					/



15.2.2. 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*!

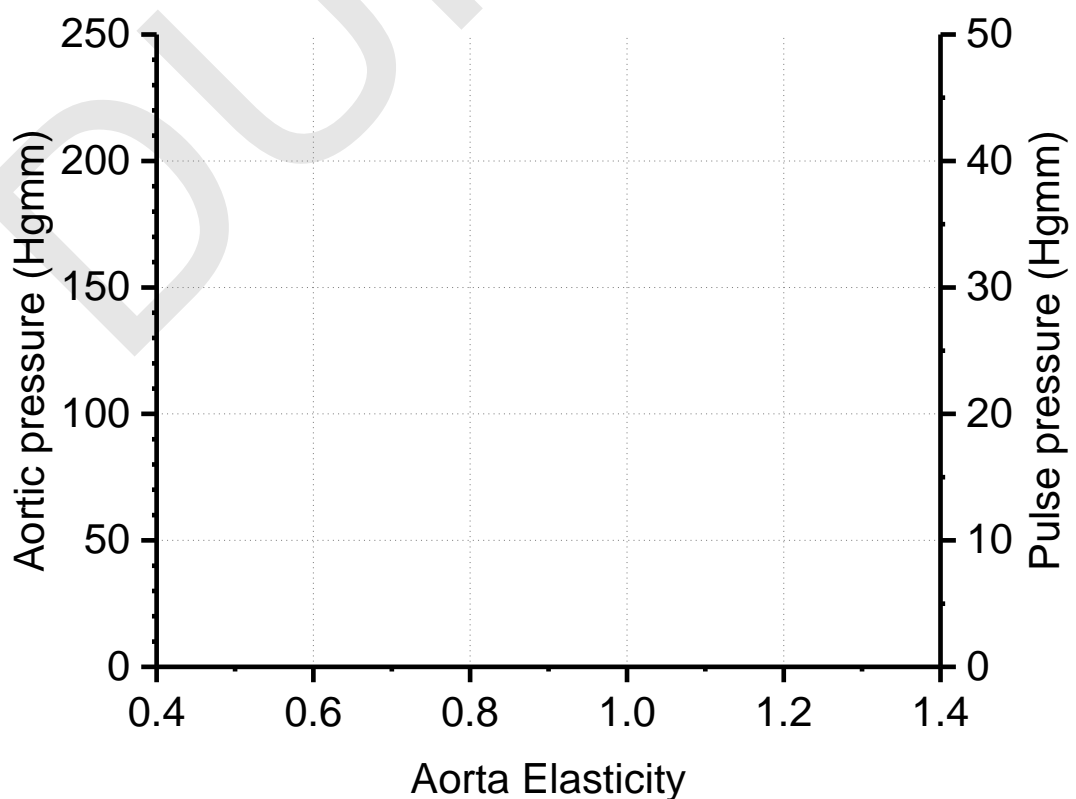
TPR (Hgmm *ms/ml)	End-diastolic volume (ml)	End-systolic volume (ml)	Stroke volume (ml)	Ejection fraction	Aortic pressure [sys/dia] (Hgmm)
500					/
600					/
700					/
800					/
900					/
1000					/
1100					/



15.2.3. 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				



15.3. DYNAMICS OF THE STARLING MECHANISM

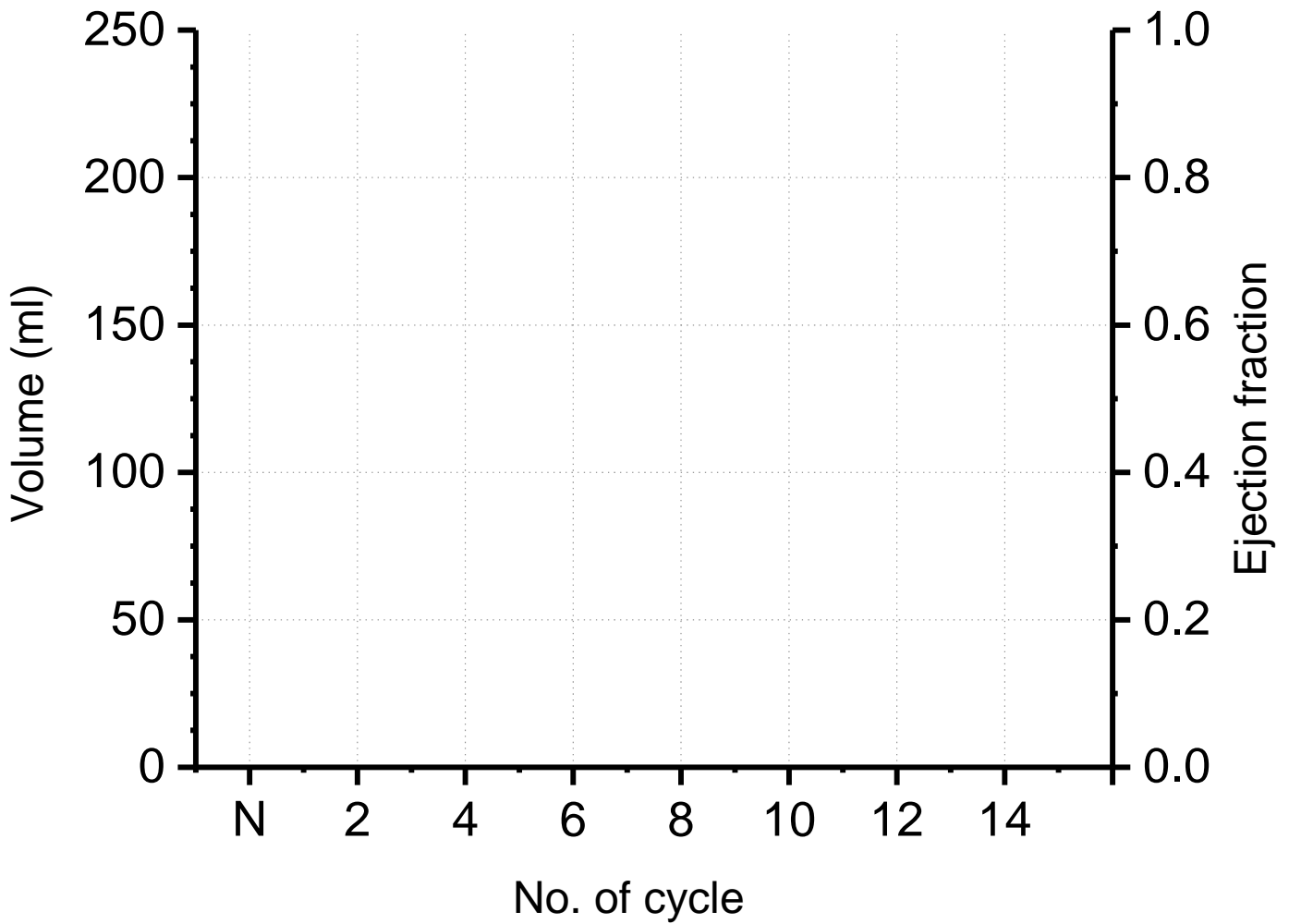
15.3.1. Role of increasing venous return

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!



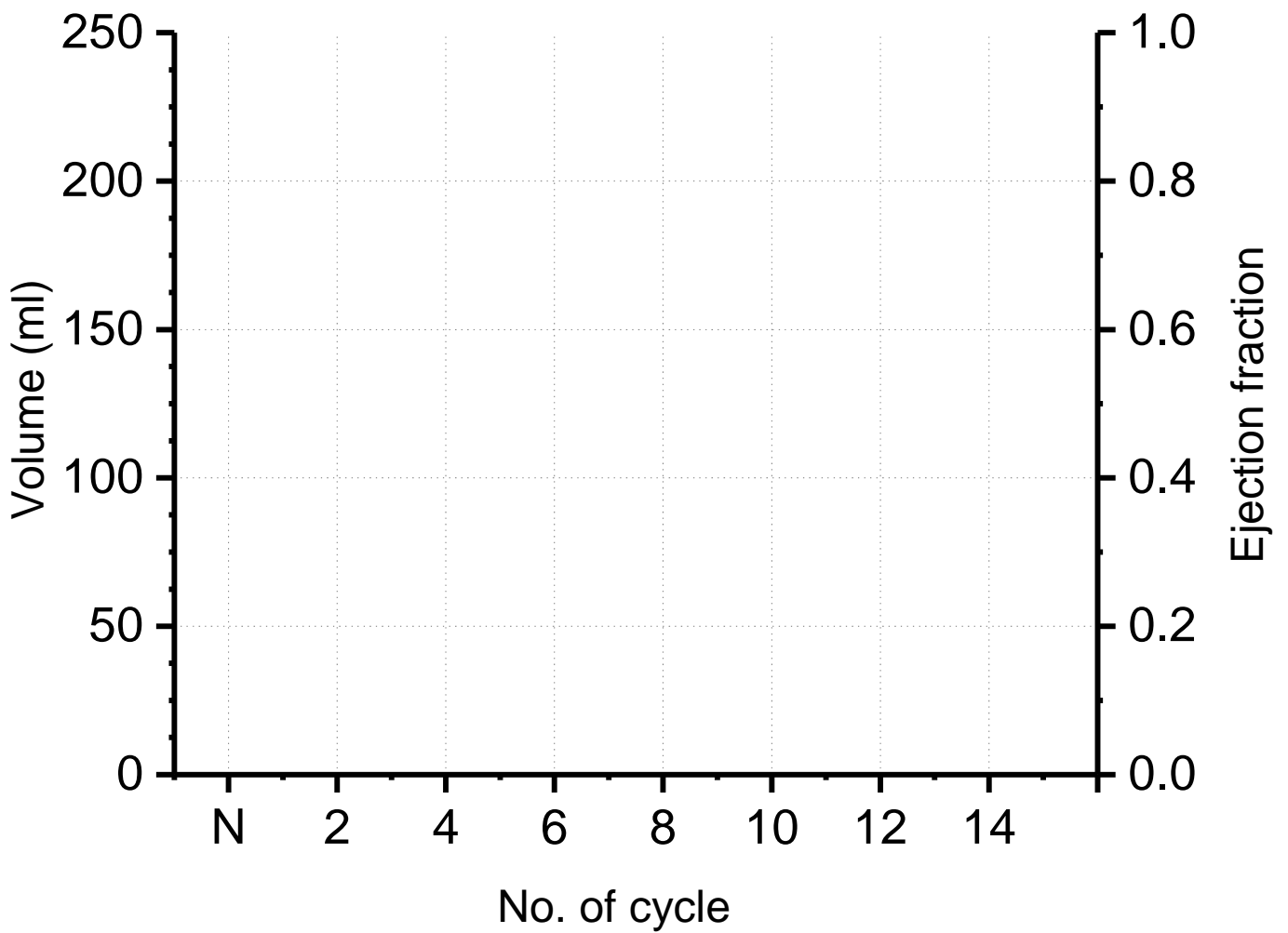
15.3.2. Role of increasing total peripheral resistance

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*!



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signature of lab teacher or helper

The lab is completed:

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date

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signature of lab teacher

TOPIC SHEET N° 16

SIMULATION OF RENAL TRANSPORT MECHANISMS

Select the desired transport mechanism from the main menu and read the information part before starting the simulation itself.

16.1. Determination of the inulin clearance

Run the simulation with the preset data. Follow the time course of the plasma inulin and the amount excreted. Calculate the inulin clearance assuming 1 mL/min for urine flow and using the initial plasma concentration (P_{IN}) and the corresponding concentration in the urine (U_{IN}).

$P_{IN} =$ $U_{IN} =$ $C_{IN} =$

Modify the initial concentration, read the corresponding excreted amount and recalculate the clearance. Repeat the calculation using at least four different P_{IN} values.

$P_{IN} =$	$P_{IN} =$	$P_{IN} =$	$P_{IN} =$
$U_{IN} =$	$U_{IN} =$	$U_{IN} =$	$U_{IN} =$
$C_{IN} =$	$C_{IN} =$	$C_{IN} =$	$C_{IN} =$

Plot C_{IN} as the function of P_{IN} .



Calculate the extraction coefficient (E) for inulin assuming a serum concentration of 0.5 mg/mL and normal 120 ml/min GFR. For the value of the filtration fraction (FF) use the numbers given below. Describe how the calculation was carried out.

FF = 0.1	ERPF =	$C_{IN} =$	$E_{IN} =$
FF = 0.2	ERPF =	$C_{IN} =$	$E_{IN} =$
FF = 0.4	ERPF =	$C_{IN} =$	$E_{IN} =$

Decrease the value of GFR and recalculate the clearance using the initial serum concentration and the corresponding excreted amount. ($V = 1$ mL/minute)

GFR =

$P_{IN} =$

$U_{IN} =$

$C_{IN} =$

16.2. Determination of the clearance of PAH

Run the program with the preset data. Note the filtered, secreted and excreted amounts of PAH as the functions of the serum PAH concentration. The assumed initial PAH concentration ($P_{PAH} = 0.3$ mg/mL) is sufficiently high to saturate the active transport processes, therefore all characteristics of PAH excretion can be demonstrated.



Give a brief description of your observations.

Choose a low initial value for P_{PAH} (e.g. 0.03 mg/mL). Determine the clearance of PAH based on the value of U_{PAH} and assuming $V = 1$ mL/min.

$$P_{PAH} = \qquad U_{PAH} = \qquad C_{PAH} =$$

Calculate the value of the renal plasma flow (RPF) using the value of 0.9 for the extraction coefficient.

$$RPF =$$

Set the initial P_{PAH} higher, and calculate the filtered, excreted and secreted amounts of PAH. Use the value of 120 mL/min for GFR. Note that in this case the secreted amount will be the same as the maximal secretion capacity of the tubules (Tm_{PAH}).

$$\begin{array}{ll}
 P_{PAH} = & \text{filtered amount} = \\
 U_{PAH} = & \text{excreted amount} = \\
 & \text{secreted amount } (Tm_{PAH}) =
 \end{array}$$

At which P_{PAH} would the filtered amount of PAH be the same as the secreted amount? In the calculation use the value of Tm_{PAH} determined above.

Decrease the secretion capacity of the tubules and determine Tm_{PAH} using the same method as above.

$$\begin{array}{ll}
 \text{Secretion capacity} = & \% \\
 P_{PAH} = & \text{filtered amount} = \\
 U_{PAH} = & \text{excreted amount} = \\
 & \text{secreted amount } (Tm_{PAH}) =
 \end{array}$$

- (A) Decrease the filtration fraction to 5% (FF) then calculate the value of ERPF at low P_{PAH} . ($E_{PAH}=0.9$)
- (B) Apply a definitely saturating plasma concentration of PAH as initial serum level, and determine the value of Tm_{PAH} under these conditions; ERPF should be the same as determined under (A).

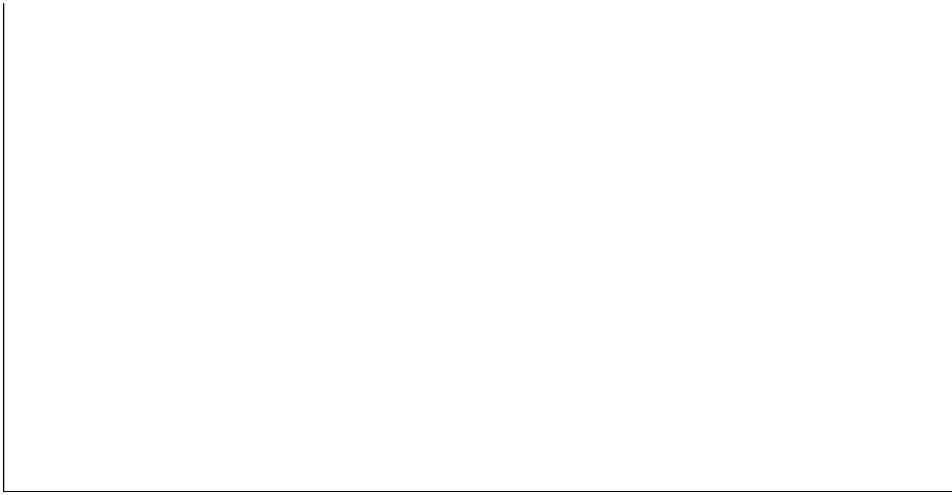
A.) $P_{PAH} =$ $C_{PAH} =$
 $U_{PAH} =$ ERPF =

B.) $P_{PAH} =$ filtered amount =
 $U_{PAH} =$ excreted amount =
secreted amount (Tm_{PAH}) =

Vary the starting plasma concentration of PAH (in the range given below) and note the corresponding excreted amounts. Calculate the clearance and the extraction coefficient using the data provided in the table (note that ERPF should be the same as determined above).

P_{PAH} (mg/mL)	U_{PAH} (mg/mL)	C_{PAH} (mL/min)	E_{PAH}
0.1			
0.2			
0.3			
0.4			
0.5			
0.6			
0.7			
0.8			
0.9			
1.0			

Plot C_{PAH} as the function of P_{PAH} .



Plot E_{PAH} as the function of P_{PAH} .



What is the theoretical minimum of C_{PAH} and E_{PAH} ?

16.3. Simulation of renal glucose transport

Choosing different concentrations of plasma glucose (P_G), plot the changes in the filtered, reabsorbed and excreted amounts of glucose as the function of P_G .



Give a brief description of your observations.

Determine T_{mG} using such a plasma glucose concentration where the transport system responsible for the glucose reabsorption is definitely saturated. Use the value of GFR in l/min (0.12 l/min).

$P_G =$ _____ filtered amount = _____

$U_G =$ _____ excreted amount = _____

GFR = _____ reabsorbed amount (T_{mG}) = _____

Decrease the reabsorption capacity and recalculate the value of T_{mG} using the previous high plasma glucose concentration. Use the value of GFR in l/min again (0.12 l/min).

Reabsorption capacity = _____ %

$P_G =$ _____ filtered amount = _____

$U_G =$ _____ excreted amount = _____

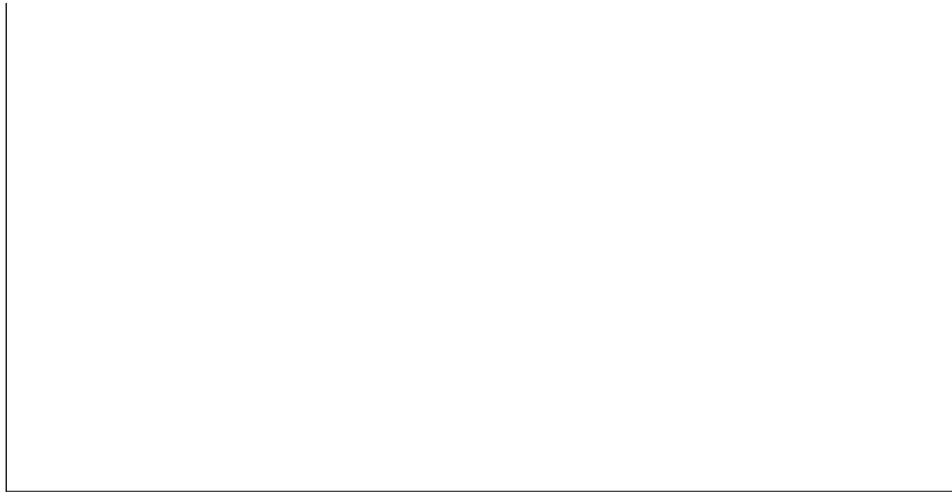
reabsorbed amount (T_{mG}) = _____

Use high enough plasma glucose concentration (20 mmol/L) to saturate the transport system responsible for the glucose reabsorption and study the effect of a lowered GFR (60 mL/min) and describe the effects of these modifications on the excreted amount of glucose.

Vary the starting plasma concentration of glucose (in the range given below), and note the corresponding excreted amounts. Using these data calculate the clearance and the extraction coefficient. (Pay attention to the units when calculating the value of clearance).

P_G (mM/L)	U_G (mM/mL)	C_G (mL/min)	E_G
5			
10			
15			
20			
25			
30			
35			
40			
45			
50			

Plot C_G as the function of P_G .



Plot E_G as the function of P_G .



What is the theoretical maximum of C_G and E_G ?

The student was present:

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signature of lab teacher or helper

The lab is completed:

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date

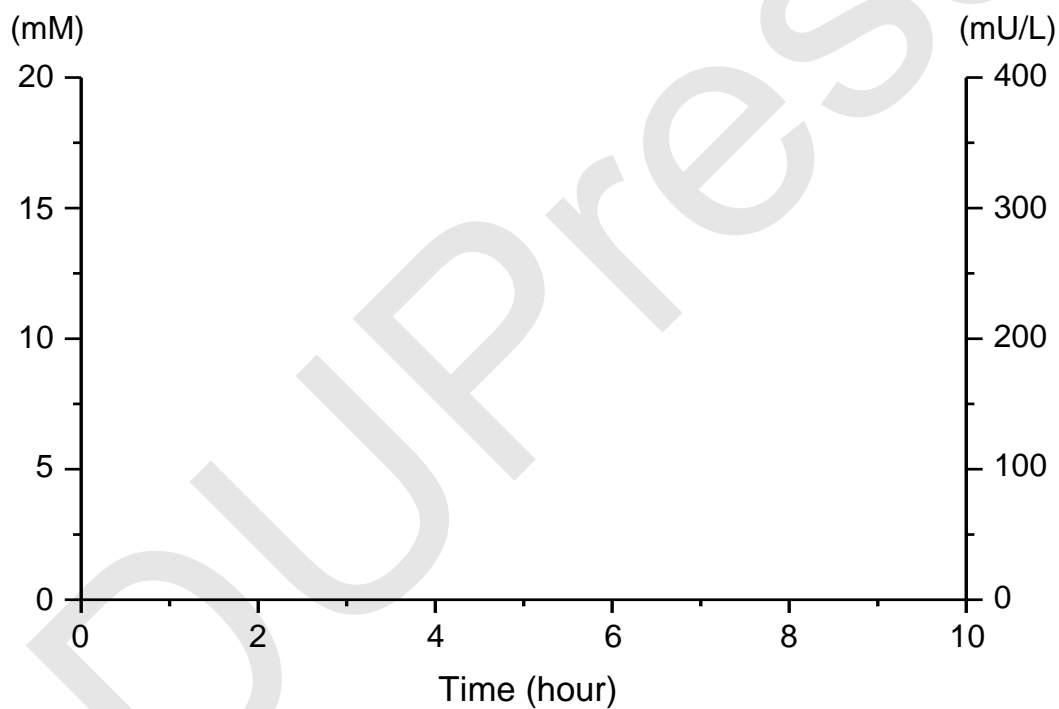
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signature of lab teacher

TOPIC SHEET N° 17

COMPUTER SIMULATION OF THE GLUCOSE TOLERANCE TEST

17.1. Response of a healthy patient to a single glucose load, the states of reduced and increased glucose tolerance.

Run the program first simulating the normal (N) situation then run it again with decreased (D) tolerance without clearing the screen in between. Draw a graph showing the levels of blood glucose and insulin as the function of time for both the normal and reduced glucose tolerance. Do not forget to indicate which is the normal and pathological curve.

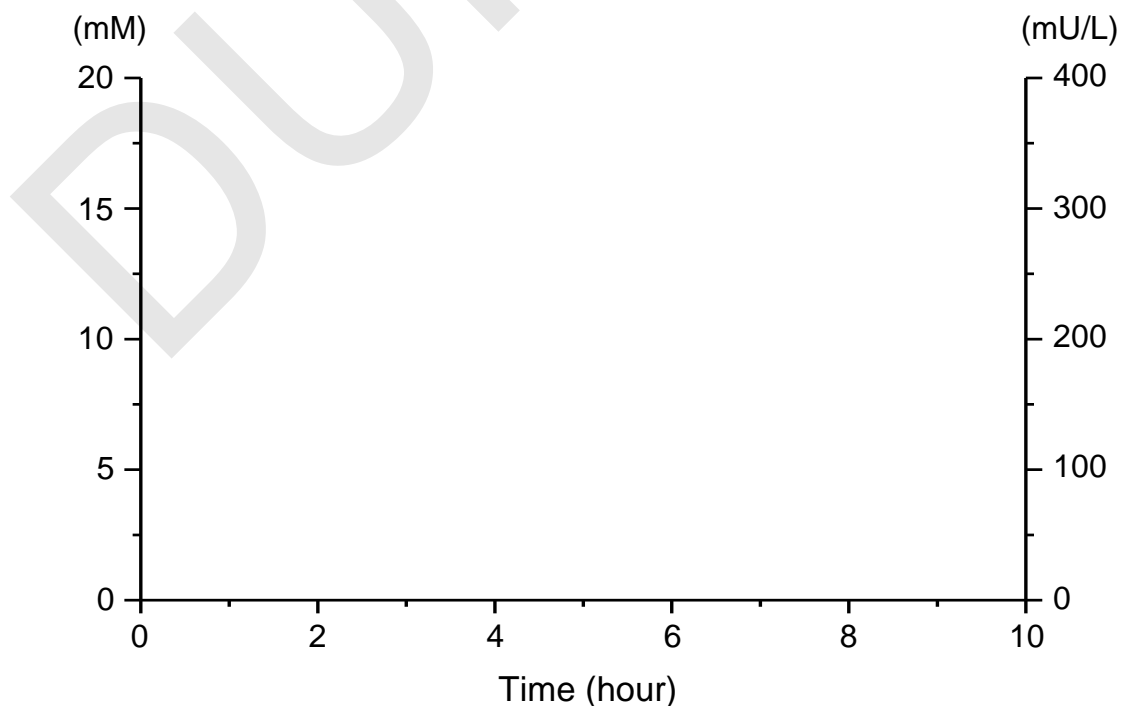


Fill out the table below using the data shown on the screen.

	normal tolerance	reduced tolerance
a. initial glucose level: mmol/L
b. glucose peak under load: mmol/L
c. glucose level at 150 min after load: mmol/L
d. glucose excreted with urine: mmol
e. maximum insulin activity: mU/L

Give the most significant differences between the reduced and normal glucose tolerance using the data from above.

Repeat the simulation using **double glucose load** by choosing the Staub-Traugott mode in the menu. Run the simulation program in the case of normal and decreased tolerance. After the normal curve is displayed, repeat the simulation **without clearing the screen** choosing the **Staub-Traugott mode** again with decreased tolerance. Make a hand-drawn copy of the screen on the graph below, showing the characteristic curves of the double glucose load. Fill out the table below using the data obtained from the program.



Study the state of increased (I) glucose tolerance using Staub-Traugott mode. Run the program first in the normal tolerance mode and after the normal curve is displayed, choose the Staub-Traugott mode again. Repeat the simulation for the increased tolerance **without clearing the screen**. Fill out the table below using the data obtained from the simulation program.

	normal tolerance	reduced tolerance	increased tolerance
a. starting glucose level:mmol/L
b. glucose peak after the first load:mmol/L
c. glucose peak after the second load:mmol/L
difference of b and c:mmol/L
d. blood glucose level 150 min after the second load:mmol/L
e. glucose excreted with urine after the first load:mmol
f. glucose excreted with urine after the second load: mmol
g. total glucose excreted: mmol
h. maximum of insulin activity during the first load: mU/L
i. maximum of insulin activity during the second load: mU/L

Compare the reaction of an individual with normal glucose tolerance to that observed in the states of both increased and reduced tolerance. Summarize the most important differences.

17.2. Investigation of the response of the body to a single glucose load with user defined parameters

17.2.1. Effect of decreased renal threshold

Select the DATA SETTING menu and decrease the renal threshold using for instance 3 and 8 mmol/L values. Evaluate the changes in a few words by comparing the observed effects to the normal situation.

17.2.2. Effect of increased liver constant

Select the DATA SETTING menu and increase the liver constant for instance to 20000 and 40000 mg/h (the liver constant cannot be more than 50000 mg/h). Evaluate the changes in a few words by comparing the observed effects to the normal situation.

17.2.3. Effect of pancreas reactivity on the glucose tolerance and on the glucose homeostasis

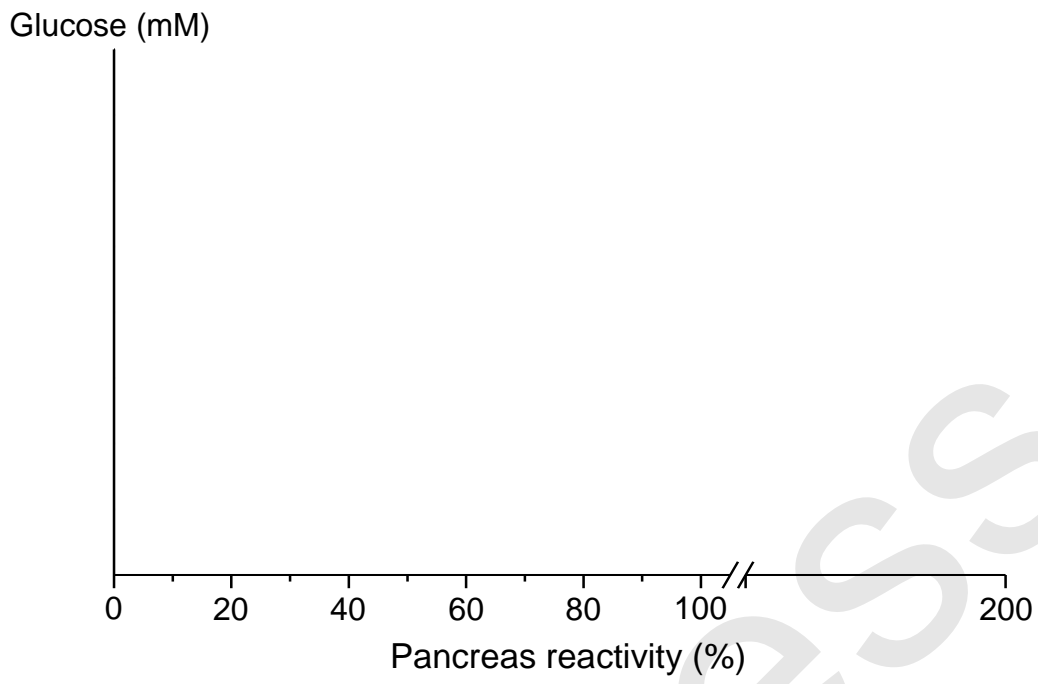
Select the DATA SETTING menu and change the relative reactivity of the pancreas to for instance 20, 50, 200 and 500% (pancreas reactivity should be at least 9% and cannot exceed 600%). Evaluate the changes in a few words by comparing the observed effects to the normal situation.

17.3. Investigation of the response of the body to a double glucose load with user defined parameters

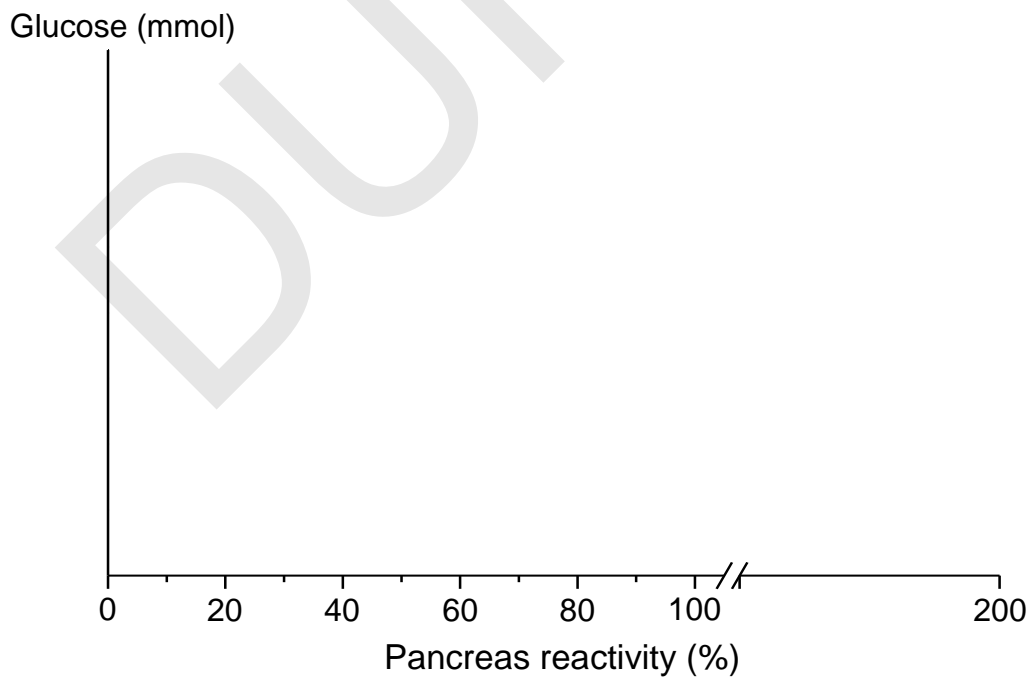
Fill out the table below by modifying the relative reactivity of the pancreas in the DATA SETTING menu. Use the double glucose load (Straub-Traugott mode)!

Pancreas reactivity (%)	glucose peak after the first load (mM)	glucose peak after the second load (mM)	glucose excreted after the first load (mmol)	glucose excreted after the second load (mmol)
10				
20				
40				
60				
80				
100				
200				

Peak of glucose



Excreted glucose



Based on the obtained graphs explain why the double glucose load method can lead to a more accurate diagnosis?

DUPress

The student was present:

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date

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signature of lab teacher or helper

The lab is completed:

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date

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signature of lab teacher

TOPIC SHEET N° 18

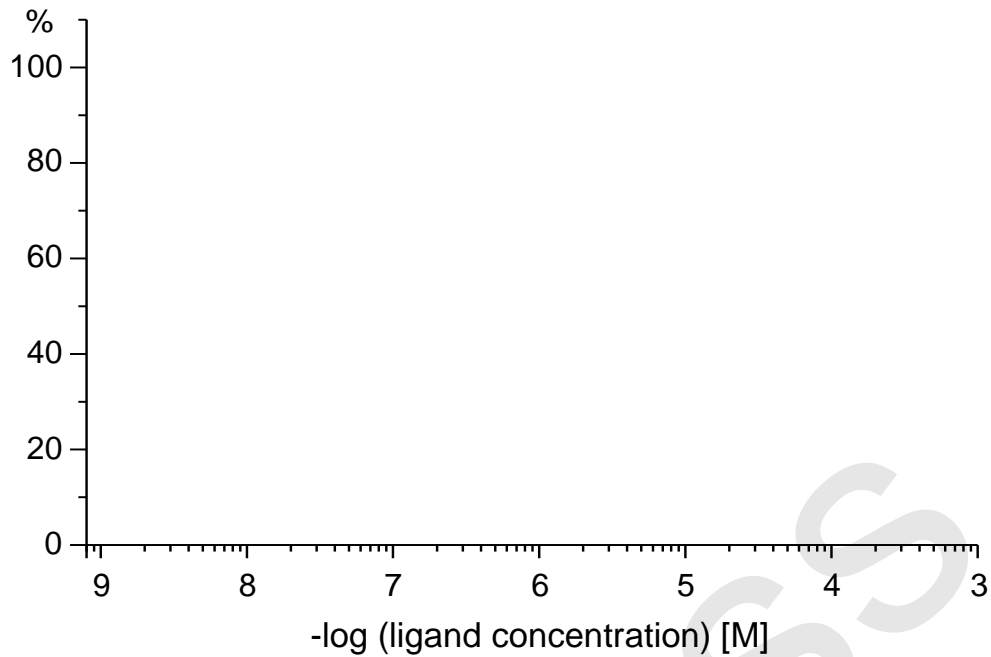
COMPUTER SIMULATION OF THE HUMORAL REGULATION OF THE
INTESTINAL SMOOTH MUSCLE

18.1. 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 $0.05 \mu\text{M}$ atropine. Plot the dose-response curve with the new data.

Repeat the experiment after replacing atropine with $0.05 \mu\text{M}$ hexamethonium. Please, do not forget to wash the preparation between the application of the antagonists.

ACh concentration ($\mu\text{mol/L}$)	Change in tension in Control		Change in tension in the presence of $0.05 \mu\text{M}$ atropine		Change in tension in the presence of $0.05 \mu\text{M}$ 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.

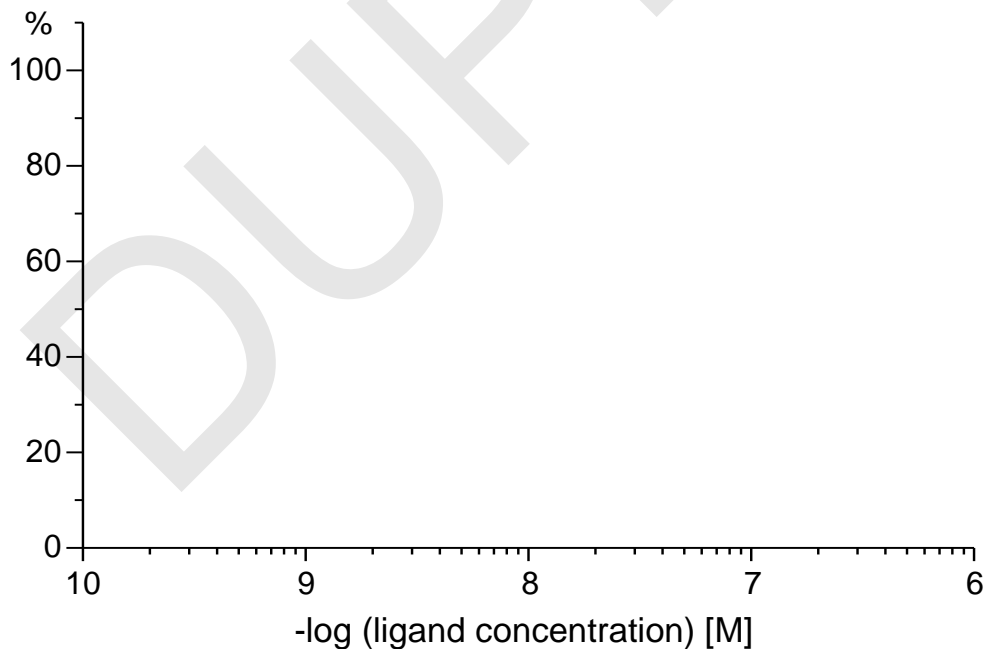
How do you explain the effect of acetylcholine? What kind of receptor is involved in the action of acetylcholine, and what second messenger pathway it is linked to?

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?

18.2. 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 ($\mu\text{mol/L}$)	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		

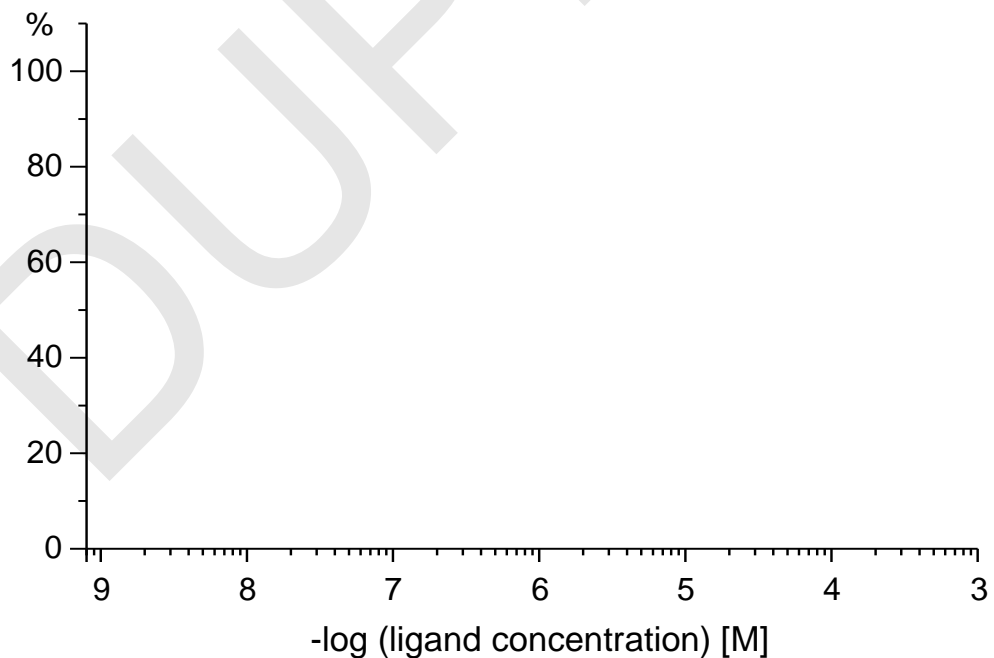


What is the half inhibitory concentration (IC_{50}) of atropine? Compare the affinities of the receptor for acetylcholine and atropine.

18.3. 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 ($\mu\text{mol/L}$)	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				

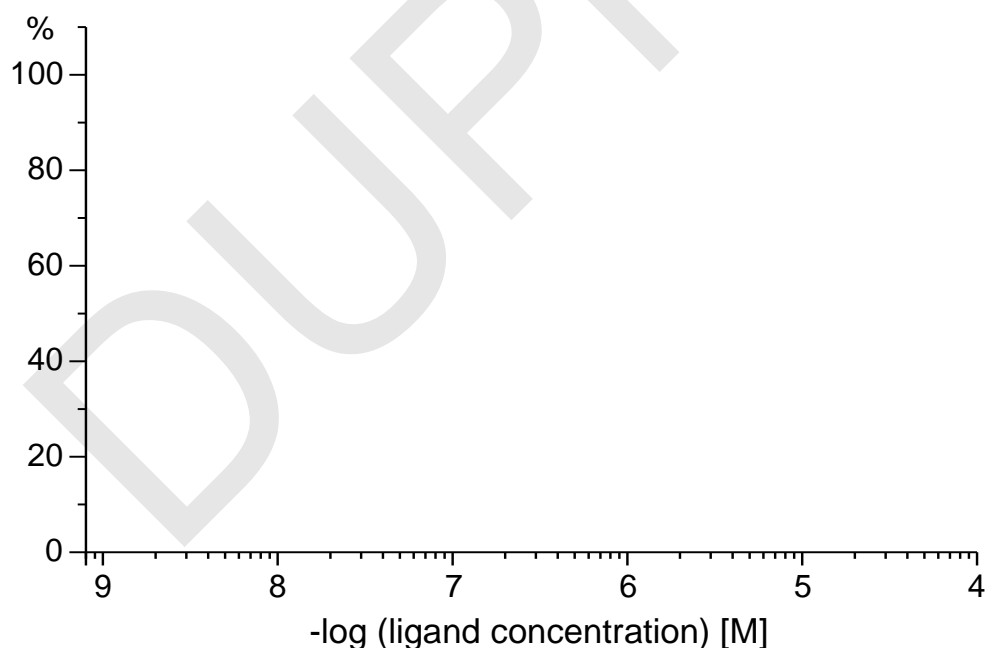


How do you explain the effect of physostigmine?

18.4. 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 ($\mu\text{mol/L}$)	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				

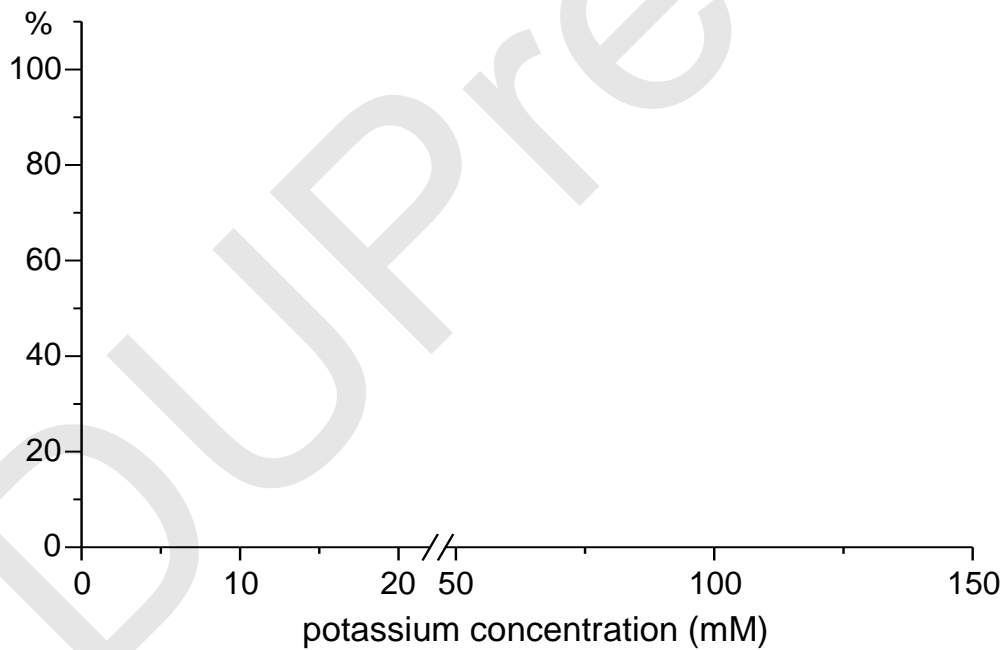


How do you explain the effect of histamine?

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

18.5. 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?

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

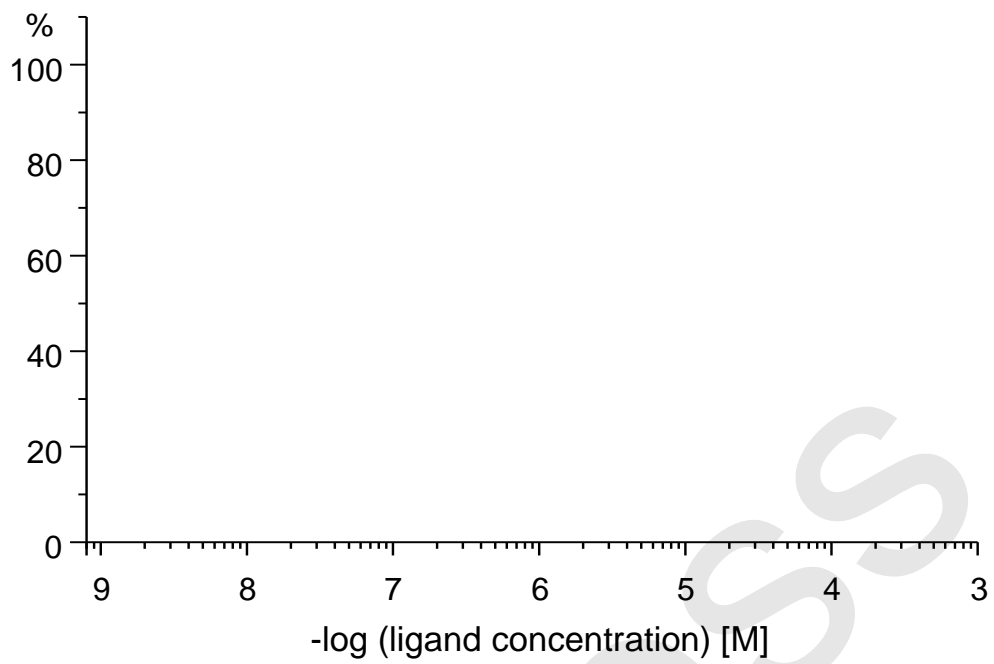
Working hypothesis:

Effects:

Results:

Ligand concentration ($\mu\text{mol/L}$)	Change in tension	
	Measured value	Normalized to the measured maximum

Dose-response curve:



Opinion:

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date

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signature of lab teacher or helper

The lab is completed:

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signature of lab teacher

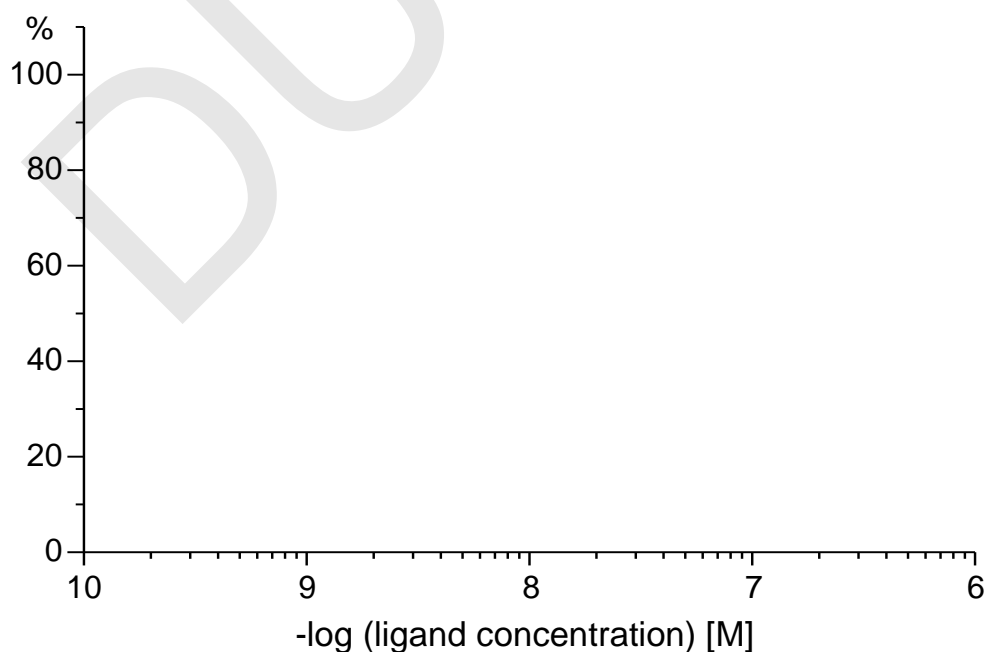
TOPIC SHEET N° 19

INVESTIGATION OF THE ENDOTHELIAL FUNCTION ON ISOLATED ARTERIAL RING

19.1. 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}).

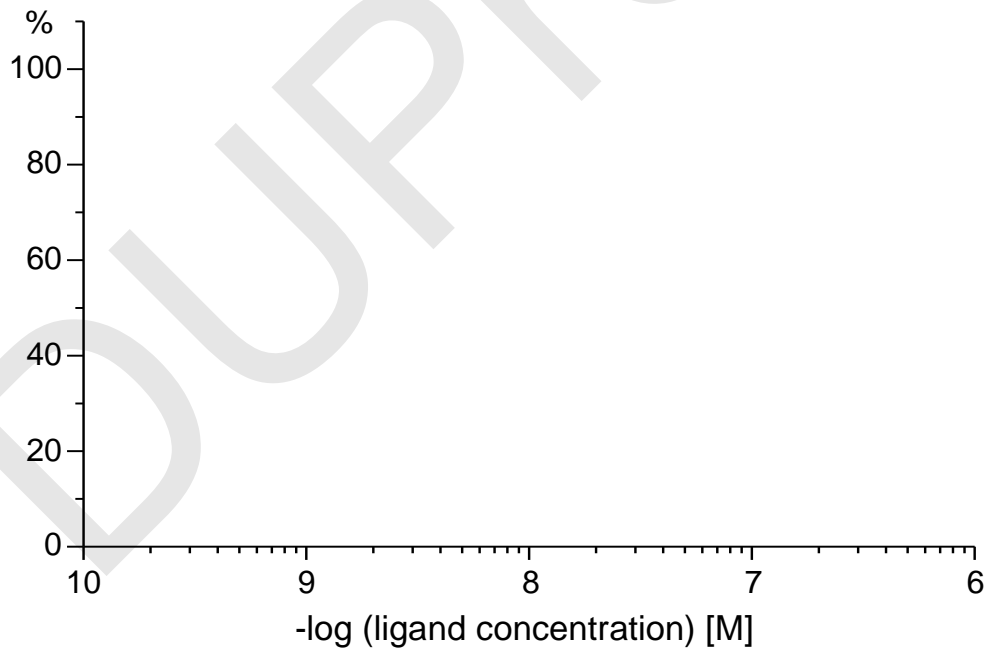
Norepinephrine concentration (mol/L)	With intact endothelium		Without endothelium	
	Measured value	Normalized to the measured maximum	Measured value	Normalized to the measured maximum
5×10^{-10}				
1×10^{-9}				
5×10^{-9}				
1×10^{-8}				
5×10^{-8}				
1×10^{-7}				
5×10^{-7}				
1×10^{-6}				



19.2. 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 (EC₅₀). (Consider the change of tension induced by L-NMMA)

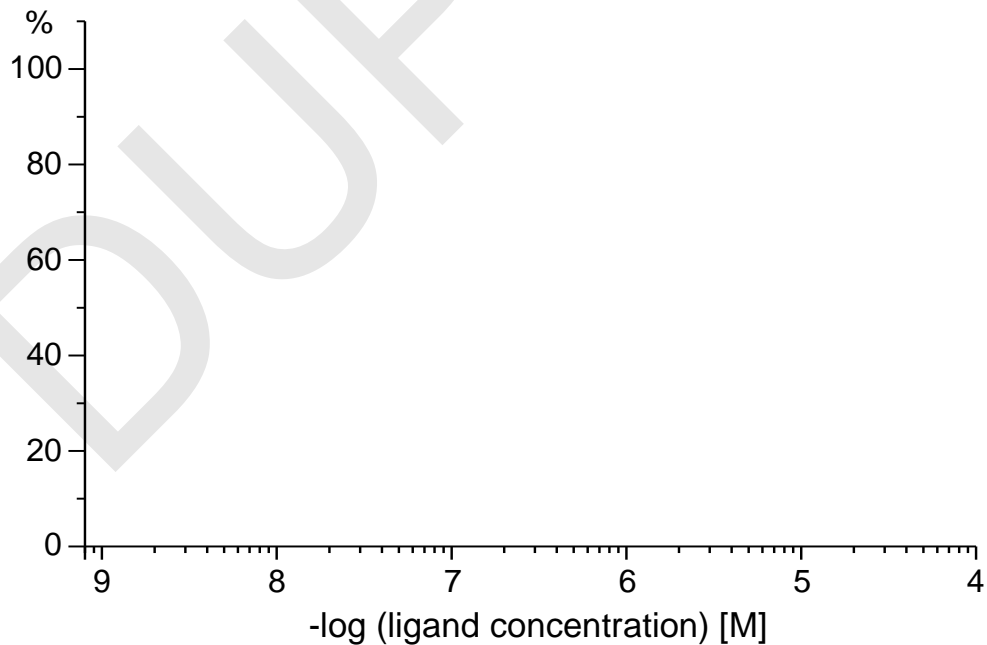
Norepinephrine concentration (mol/L)	With intact endothelium in the presence of L-NMMA		Without endothelium in the presence of L-NMMA	
	Measured value	Normalized to the measured maximum	Measured value	Normalized to the measured maximum
5 x 10 ⁻¹⁰				
1 x 10 ⁻⁹				
5 x 10 ⁻⁹				
1 x 10 ⁻⁸				
5 x 10 ⁻⁸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



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

19.3. 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 5×10^{-7} mol/L norepinephrine. The endothelium free arterial ring preparation does not require norepinephrine to develop spontaneous tensions.

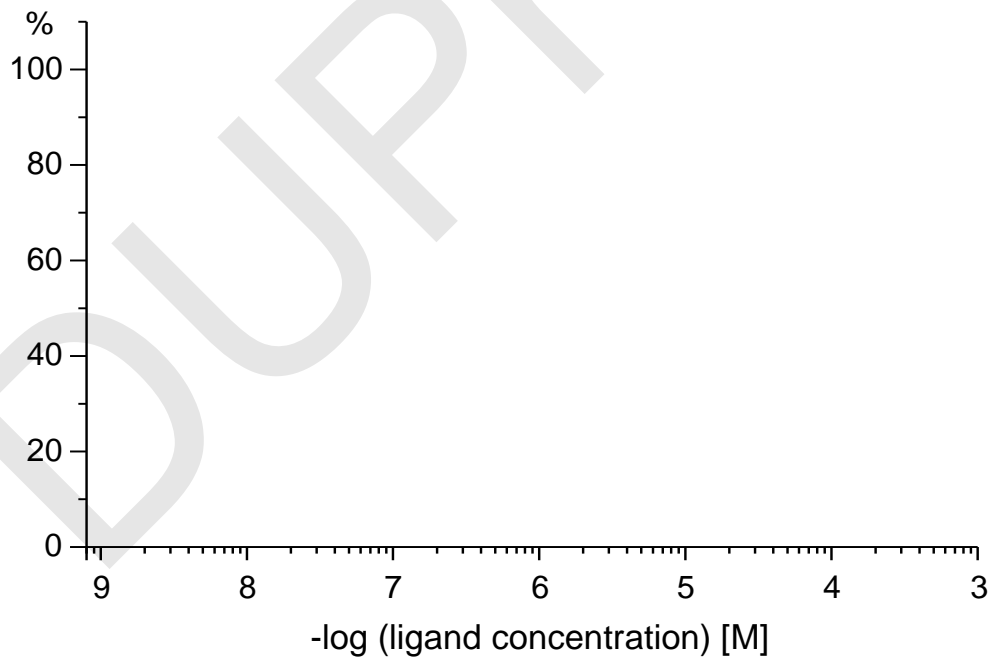
Acetylcholine concentration (mol/L)	With intact endothelium in the presence of norepinephrine		Without endothelium	
	Measured value	Normalized to the measured maximum	Measured value	Normalized to the measured maximum
0				
1×10^{-9}				
5×10^{-9}				
1×10^{-8}				
5×10^{-8}				
1×10^{-7}				
5×10^{-7}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



How do you explain the results?

19.4. 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. 5×10^{-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.

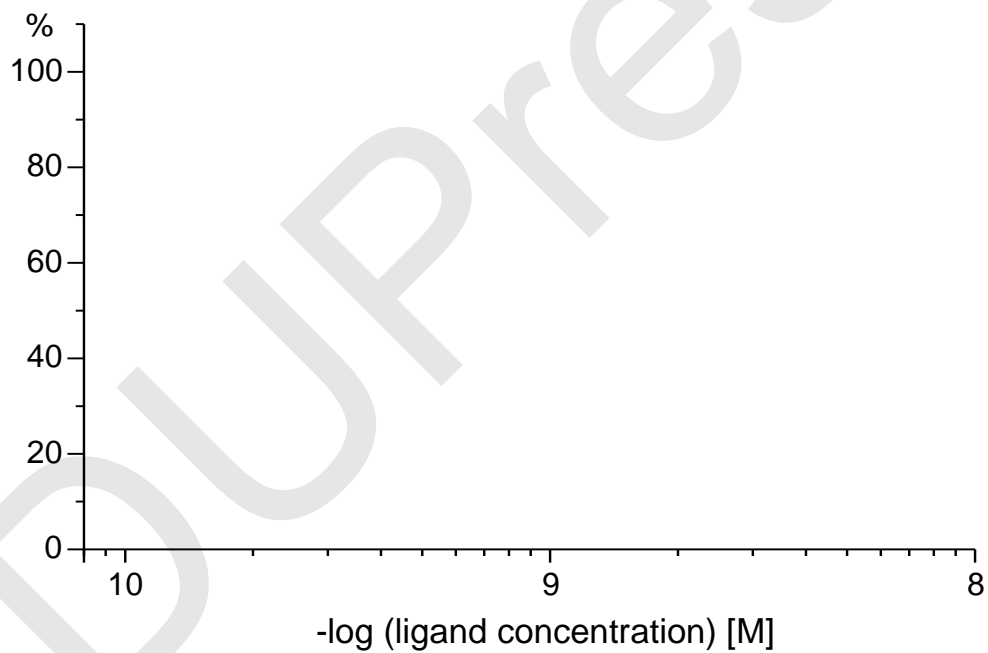
Acetylcholine concentration (mol/L)	Antagonist concentration		Antagonist concentration	
	Measured value	Normalized to the measured maximum	Measured value	Normalized to the measured maximum
0				



How do you explain the results?

19.5. 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?

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

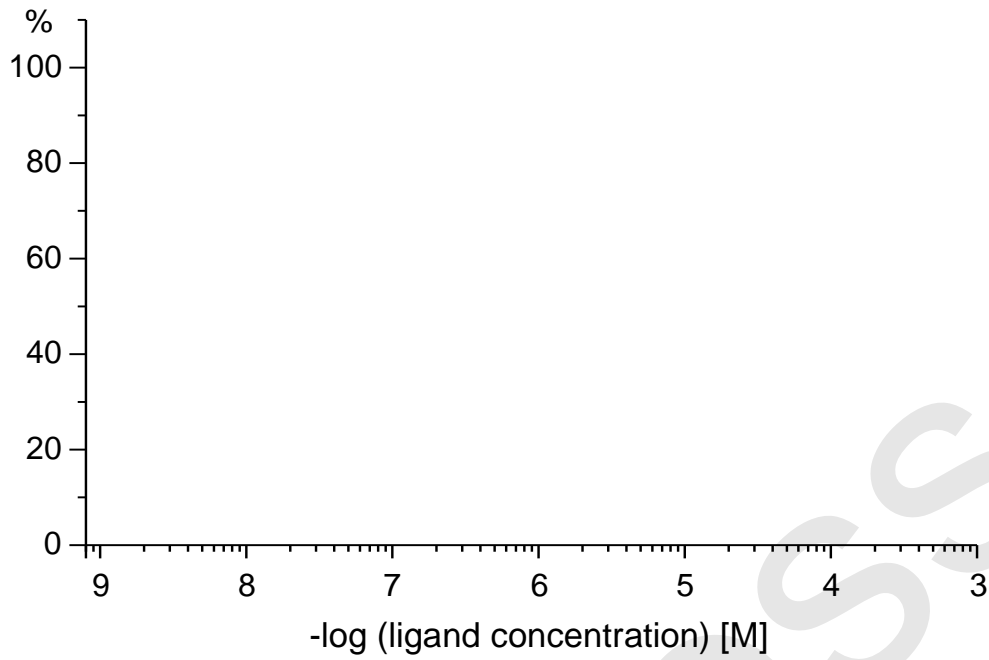
Working hypothesis:

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° 20

COMPUTER SIMULATION OF THE SKELETAL MUSCLE FUNCTION

20.1. Stimulus-dependent force generation

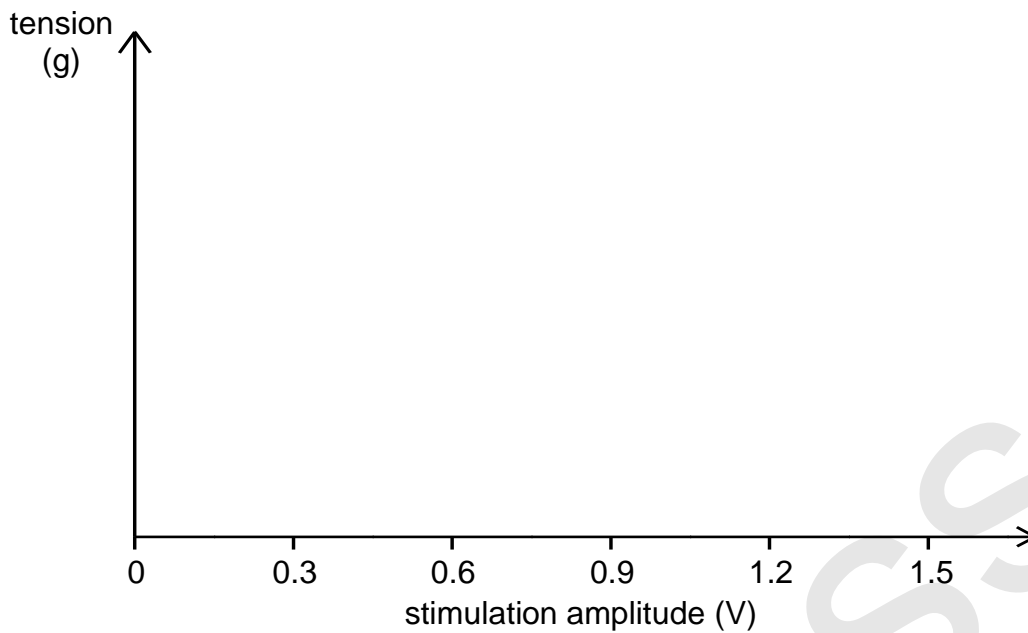
Choose the appropriate menu point in the “*Skeletal Muscle Function*” simulation program and examine the skeletal muscle tension using stimulation amplitude between 0 and 1.6 V!

stimulation amplitude (V)	tension (g) – “Amp” in the program
1. 0
2. 0.1
3. 0.2
4. 0.3
5. 0.4
6. 0.5
7. 0.6
8. 0.7
9. 0.8
10. 0.9
11. 1.0
12. 1.1
13. 1.2
14. 1.3
15. 1.4
16. 1.5
17. 1.6

Draw a representative muscle contraction!



Plot the values of muscle tension as the function of the stimulation amplitude applied!



Answer the following questions!

In which part of the spinal cord are the motoneurons located?

What is the name of the individual skeletal muscle cells?

What is the name of the functional unit formed by the a single motoneuron together with the innervated muscle fibres?

What is the consequence of a single action potential conducted in the motor unit?

What is the explanation of the fact that small amplitude stimuli cannot evoke muscle contraction on the examined preparation?

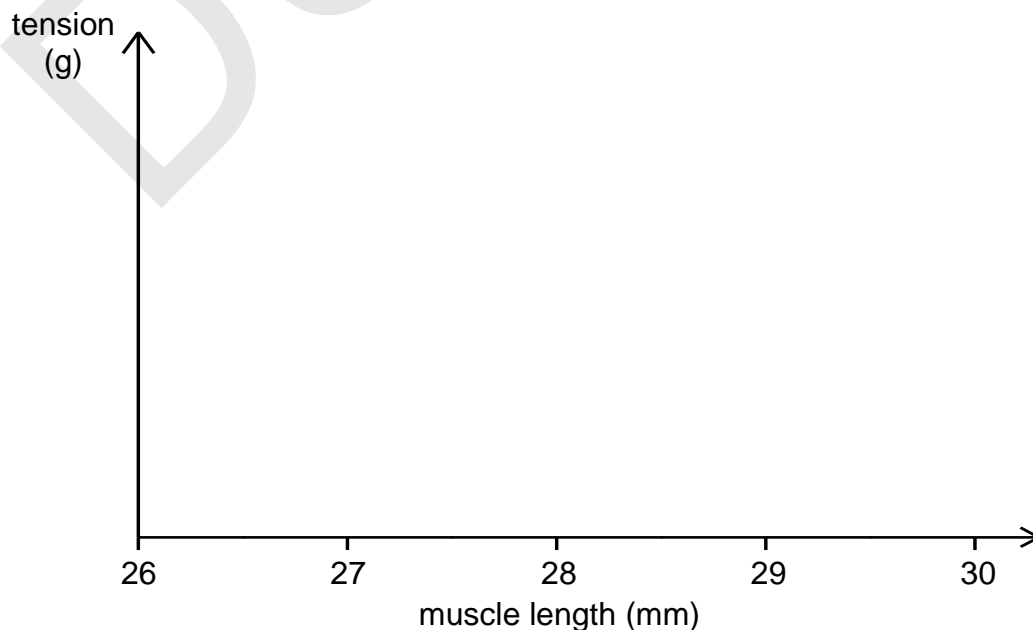
How do you explain the increased tension occurring when the amplitude of the stimulation is increased?

Even though the stimulus amplitude is continuously increased, the tension will not increase above a certain limit. What is the explanation of this phenomenon?

20.2. Length-tension relationship

Choose the appropriate menu point in the “*Skeletal Muscle Function*” simulation program and determine and plot the skeletal muscle tension between 26.0 and 30.0 mm muscle lengths using 1.5 V stimulation amplitude!

muscle length (mm)	tension (g)
1. 26.0
2. 26.5
3. 27.0
4. 27.5
5. 28.0
6. 28.5
7. 29.0
8. 29.5
9. 30.0



Answer the following questions!

What structure are calcium ions released from by the action potential reaching the transversal- (T-) tubules?

What these calcium ions bind to?

What kind of conformational change is induced by the bound calcium? What structure is affected by the calcium binding?

What kind of relationship exists between the number of cross-bridges within the muscle and the degree of muscle tension?

How does the muscle elongation affect the sarcomere length?

What could be the basal (resting) length of the frog gastrocnemius muscle?

20.3. Principles of summation and tetanus

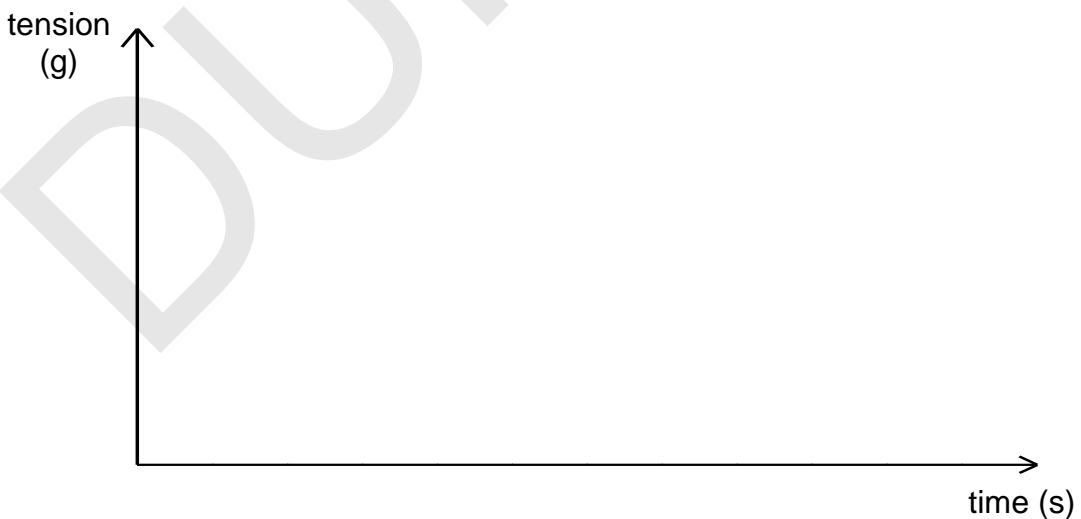
Choose the appropriate menu point in the “*Skeletal Muscle Function*” simulation program and examine the effects of different interpulse intervals using consecutive stimulation (amplitude: 1,5 V) on the skeletal muscle tension!

Draw a scenario when two consecutive stimuli produce summation!



What is the longest interpulse interval causing summation?

Draw a scenario when two consecutive stimuli produce incomplete tetanus!



What is the longest interpulse interval resulting in incomplete tetanus?

Draw a scenario when consecutive stimuli produce complete tetanus!



What is the longest interpulse interval resulting in complete tetanus?

Answer the following questions!

What is the relation between the number of motoneurons and the number of innervated muscle fibers in the mammalian skeletal muscle?

How does the intracellular Ca^{2+} -concentration increase after the action potential in the skeletal muscle?

What are the time courses of the intracellular Ca^{2+} -concentration increase and the evoked tension?

How does the tension of an individual twitch relate to that measured during tetanus?
What is your conclusion from this regarding the intracellular Ca^{2+} -concentration?

What conclusion can be drawn regarding the Ca^{2+} -movement between the sarcoplasmic reticulum and cytoplasm on the basis of the interpulse interval needed for the generation of an incomplete tetanus?

What is the reason of the slower relaxation seen after the tetanus compared with the relaxation after a simple twitch?

The student was present:

.....
date

.....
signature of lab teacher or helper

The lab is completed:

.....
date

.....
signature of lab teacher

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