UNIVERSITY OF DEBRECEN
FACULTY OF MEDICINE
DEPARTMENT OF PHYSIOLOGY

PHYSIOLOGICAL PRACTICES

for Pharmacy and Molecular Biology Students

UNIVERSITY OF DEBRECEN
FACULTY OF MEDICINE
DEPARTMENT OF PHYSIOLOGY

PHYSIOLOGICAL PRACTICES

for Pharmacy and Molecular Biology students

Debreceni Egyetemi Kiadó
Debrecen University Press

2019

Written by:

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

Language editors:

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

Editor:

Balázs Horváth, M.D., Ph.D.

Publisher:

László Csernoch, Ph.D., D.Sc.

ISBN 978-963-318-824-8

https://dupress.unideb.hu

Kiadta a Debreceni Egyetemi Kiadó Debrecen University Press
Felelős kiadó: Karácsony Gyöngyi
Készült a DE sokszorosítóüzemében, 2019-ben

Name:.....

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

The semester is verified:

…….. .....

date signature of tutor

TOPIC SHEET N^{o} 1

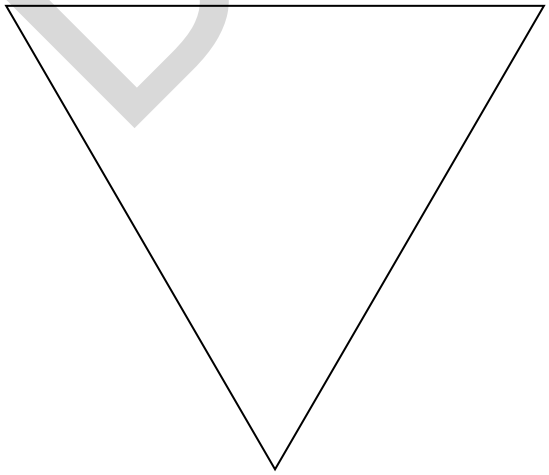
INVESTIGATION OF THE CARDIOVASCULAR FUNCTIONS

1.1. Make ECG recordings from one of your colleagues 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. How would you explain your findings?

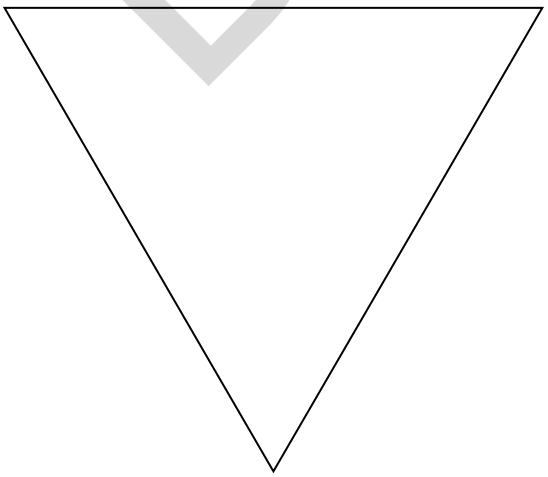
A)



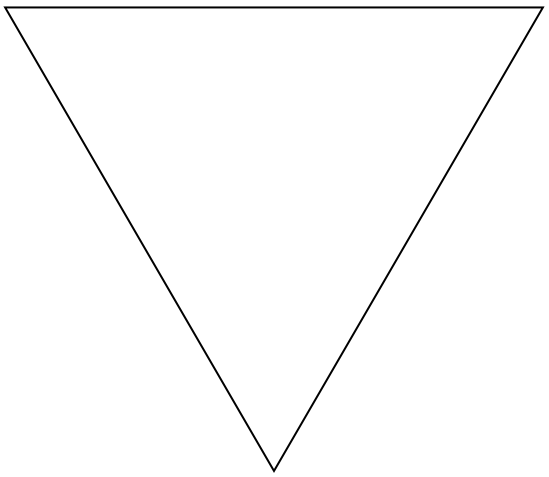
1.3. Evaluate two ECG recordings provided by your tutor, according to the criteria listed in the Practical guide. Make drawings of the characteristic parts of the recordings. What may cause such ECG alterations?

Construct the R vectors using the triangles below. How would you explain your findings?

A)



B)



1.4. 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.

1.5. 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.

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

1.7. 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.

The student was present:

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

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 one of your colleagues, 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)	

Summarize and explain your findings.

2.2. Determine the metabolic rate of the subject 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):		

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)		

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

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

The student was present:

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

The lab is completed:

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

TOPIC SHEET N^{o} 3

EXAMINATION OF THE BLOOD

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

Carry out the experiments using samples N^{o} …… 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:

…….. ……..

date signature of lab teacher or helper

The lab is completed:

…….. .....

date signature of lab teacher

TOPIC SHEET N^{o} 4

COMPUTER AIDED ACQUISITION AND PROCESSING OF BIOLOGICAL SIGNALS

4.1. 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.

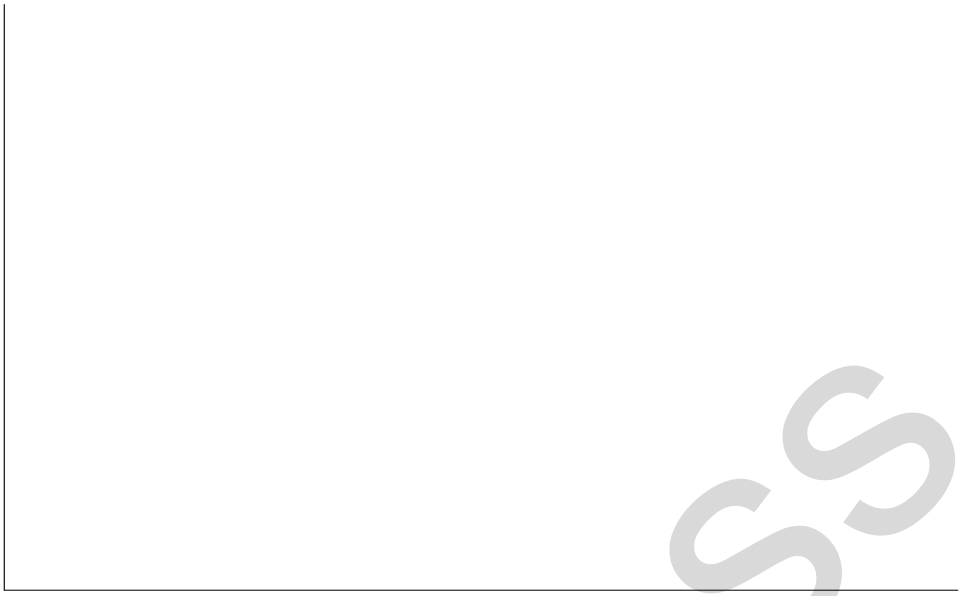
After completing the measurement, stop the data acquisition by pressing **Stop measurement** button. Save the data in a file (e.g. **CALIBRAT.DAT**). In the file name please use only letters and numbers without space or any extra symbol. You can change the time resolution or scroll the contents of the 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.

4.2. 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.

The student was present:

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

The lab is completed:

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

TOPIC SHEET N^{o} 5

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 4, before starting the data evaluation.

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

RECORD:
File name: **Gain:**

5.2. 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)			

5.3. 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)				

The student was present:

…….. ……..

date signature of lab teacher or helper

The lab is completed:

…….. .....

date signature of lab teacher

TOPIC SHEET N^{o} 6

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 4, before starting the data evaluation.

6.1. Effects of neurotransmitters and their antagonists

6.1.1. Record the contractile pattern of the uterine stripe in Tyrode solution then add 50 µL of **epinephrine** solution (Tonogen ampoule, containing 1 mg/mL epinephrine). After recording the effect of epinephrine, add 50 µL 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:**

6.1.2. After the recovery of the normal activity of the preparation, add 100 µL of **acetylcholine** solution (10 µg/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)			

6.1.3. 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:**

Measure the following parameters of the recorded smooth muscle contractions:

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

The student was present:

…….. ……..

date signature of lab teacher or helper

The lab is completed:

…….. .....

date signature of lab teacher

TOPIC SHEET N^{o} 7

SIMULATION OF THE CARDIAC CYCLE AND THE STARLING MECHANISM

7.1. EVENTS OF THE CARDIAC CYCLE

7.1.1. 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?

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

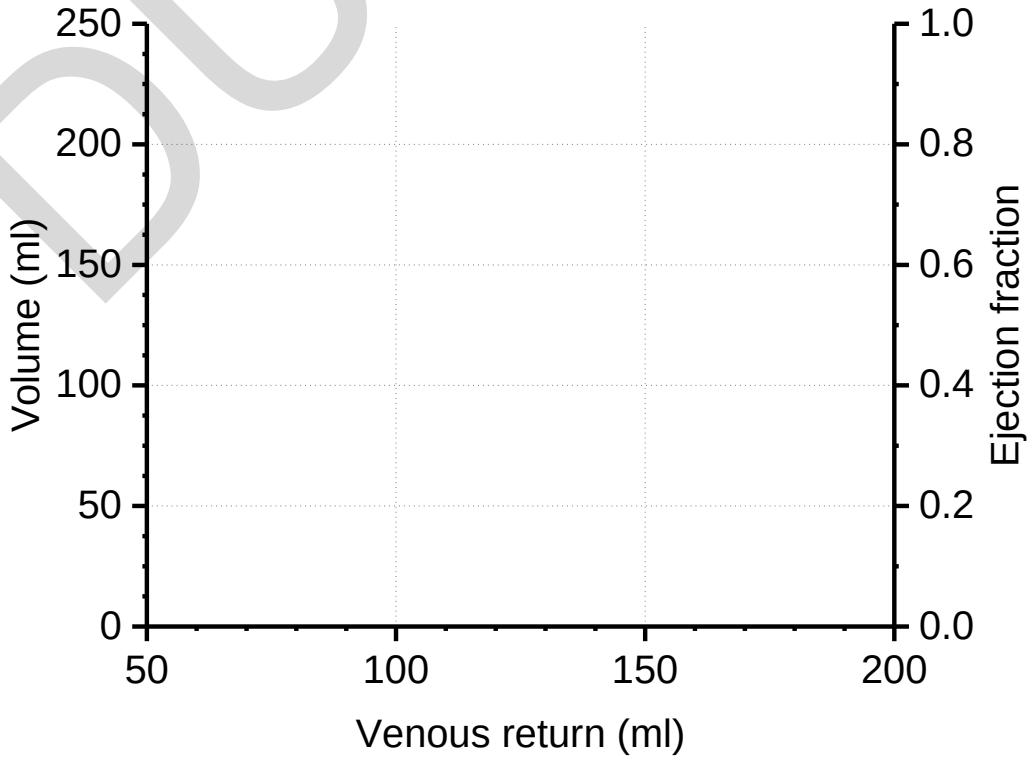
Draw and analyze how **pressure** and **outflow** changes in time within the **left ventricle** and within the **aorta**! Use maximal time resolution (0.5 s)! Indicate on the graphs where you expect sound effects to occur and explain these!

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

7.2.1. 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*!

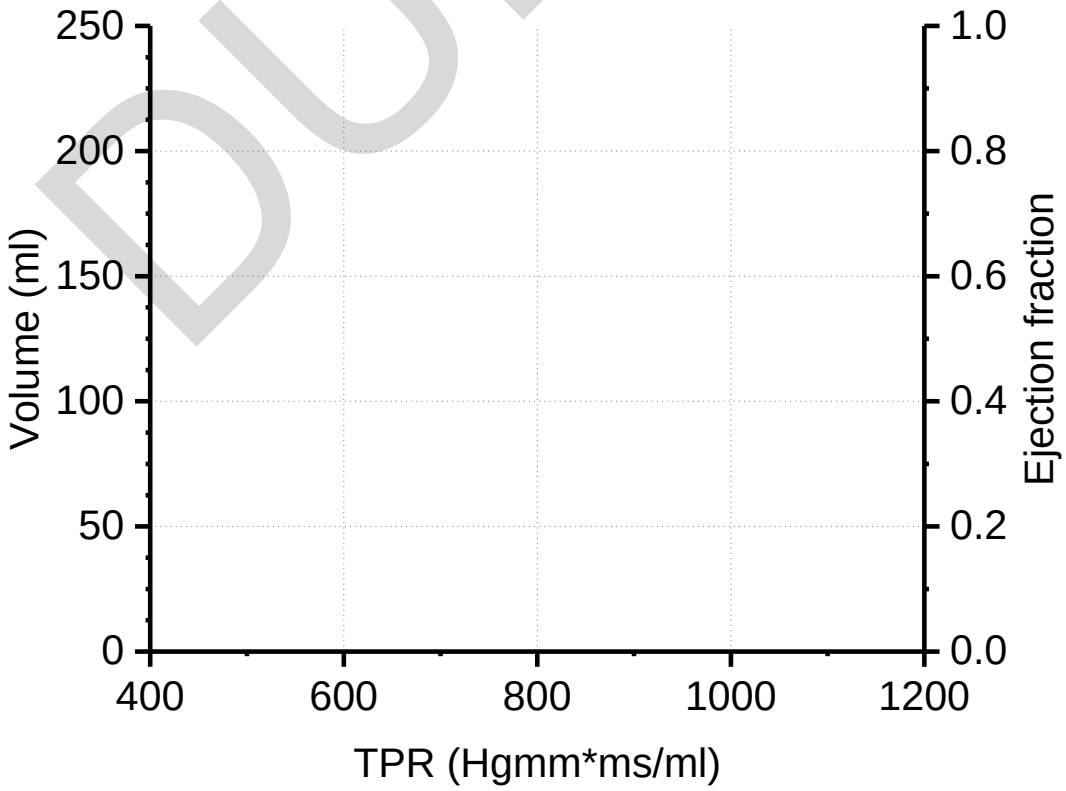
Venous return (ml)	End-diastolic volume (ml)	End-systolic volume (ml)	Stroke volume (ml)	Ejection fraction	Aortic pressure [sys/dia] (Hgmm)
60					/
80					/
100					/
120					/
140					/
160					/



7.2.2. 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					/



7.3. DYNAMICS OF THE STARLING MECHANISM

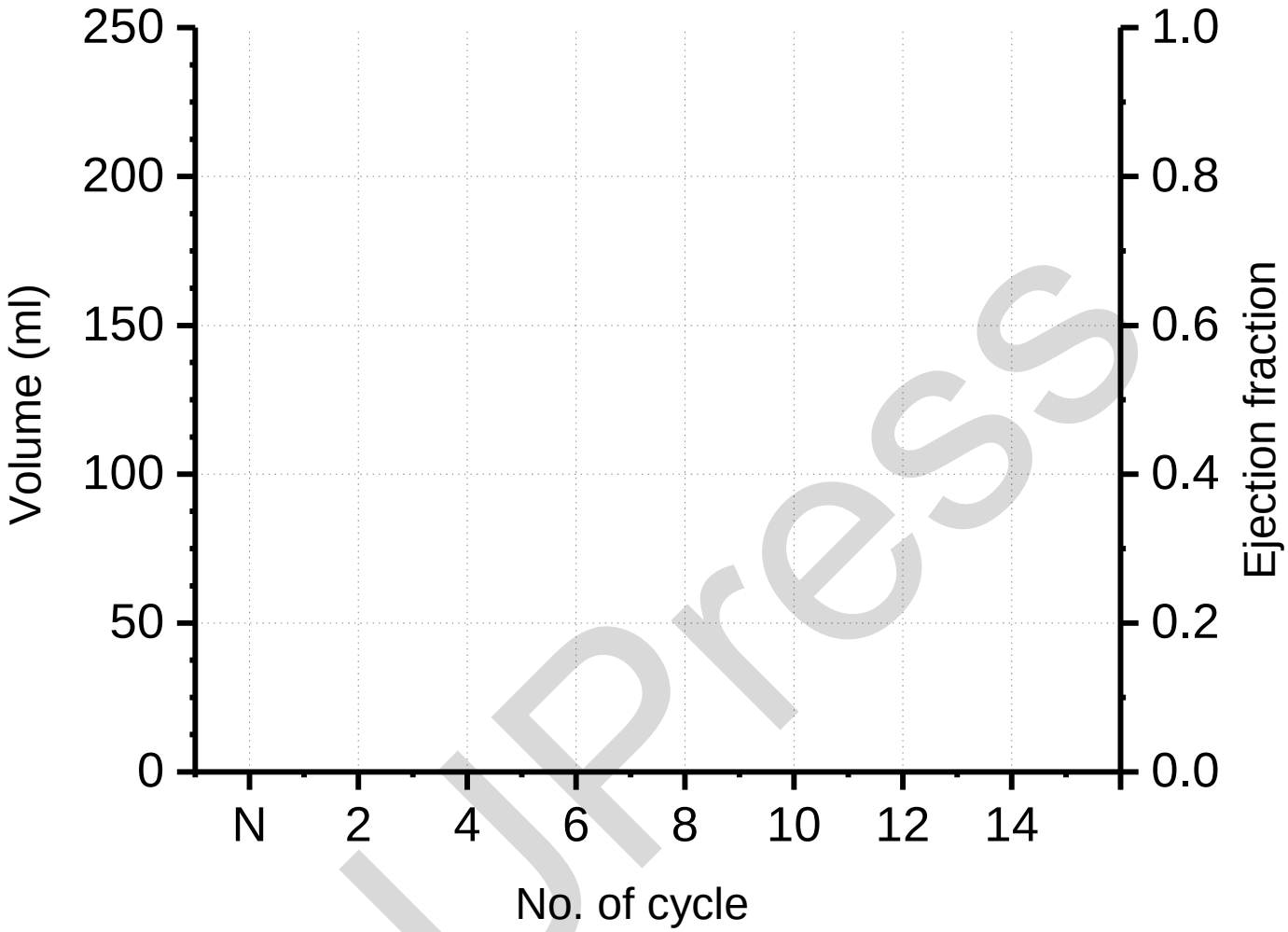
7.3.1. Role of increasing venous return

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*!



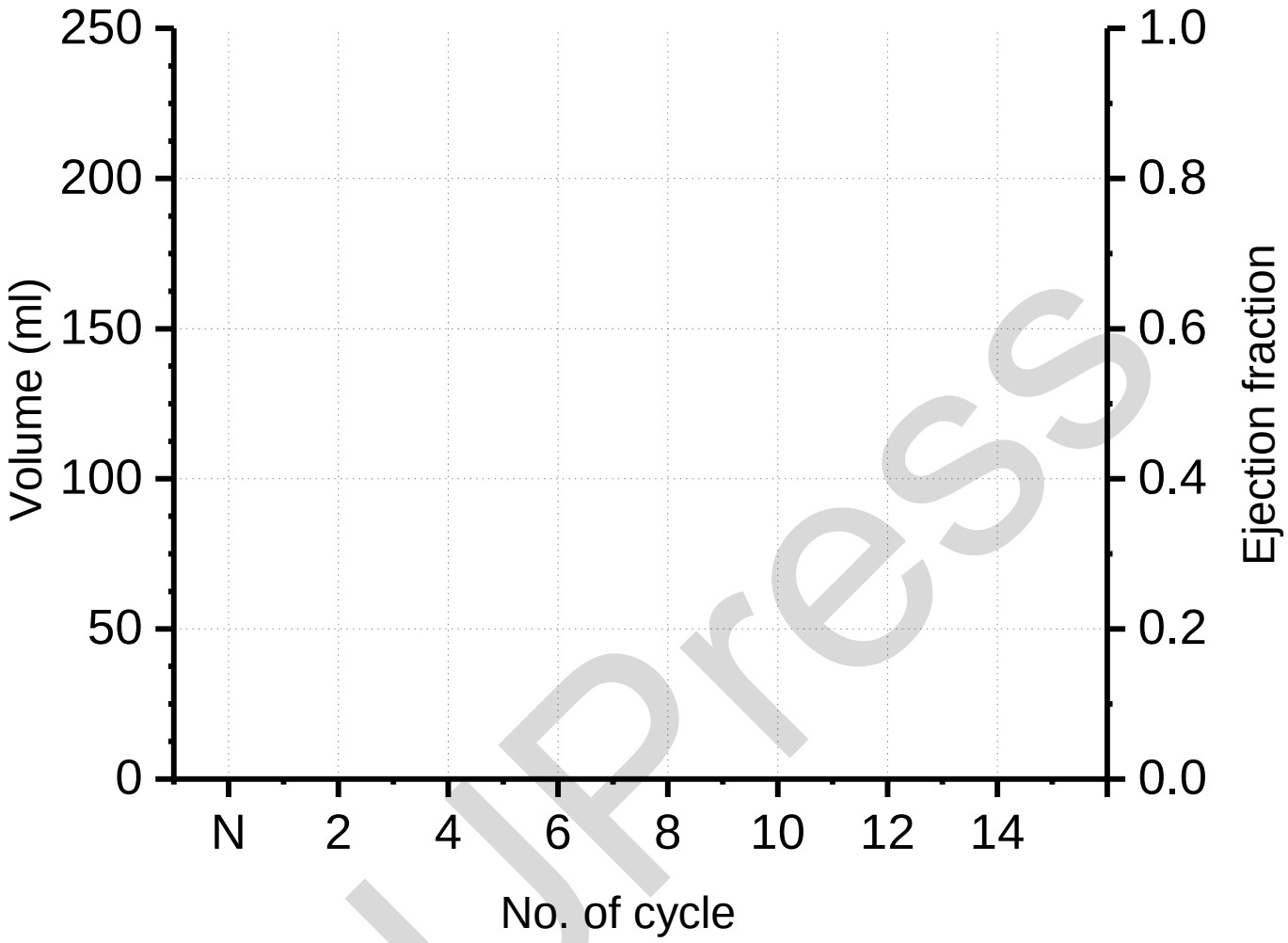
7.3.2. Role of increasing total peripheral resistance

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

TOPIC SHEET N^{o} 8

SIMULATION OF RENAL TRANSPORT MECHANISMS

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

8.1. Determination of the inulin clearance

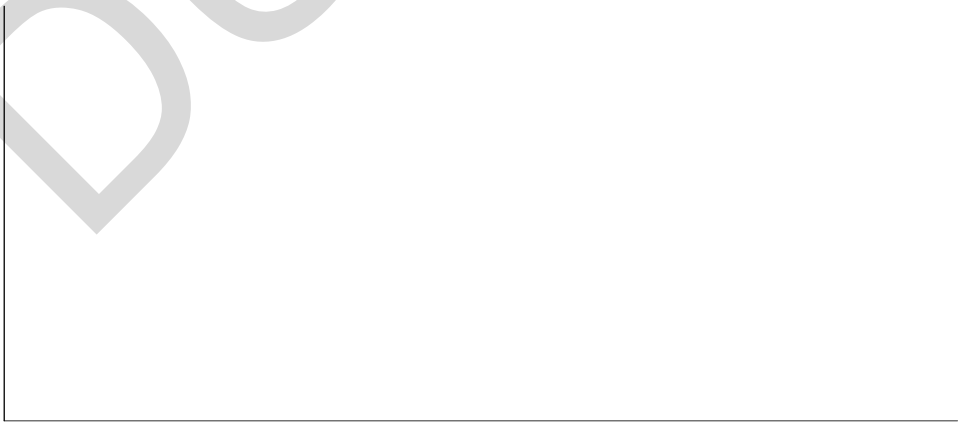
Run the simulation with the preset data. Follow the time course of the plasma inulin and the amount excreted. Calculate the inulin clearance assuming 1 mL/min for urine flow and using the initial plasma concentration (P_{IN}) and the corresponding concentration in the urine (U_{IN}).

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} =

8.2. 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} = U_{PAH} = 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}).

P_{PAH} = filtered amount =

U_{PAH} = excreted amount =

secreted amount (Tm_{PAH}) =

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.

Secretion capacity = %

P_{PAH} = filtered amount =

U_{PAH} = excreted amount =

secreted amount (Tm_{PAH}) =

(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 =

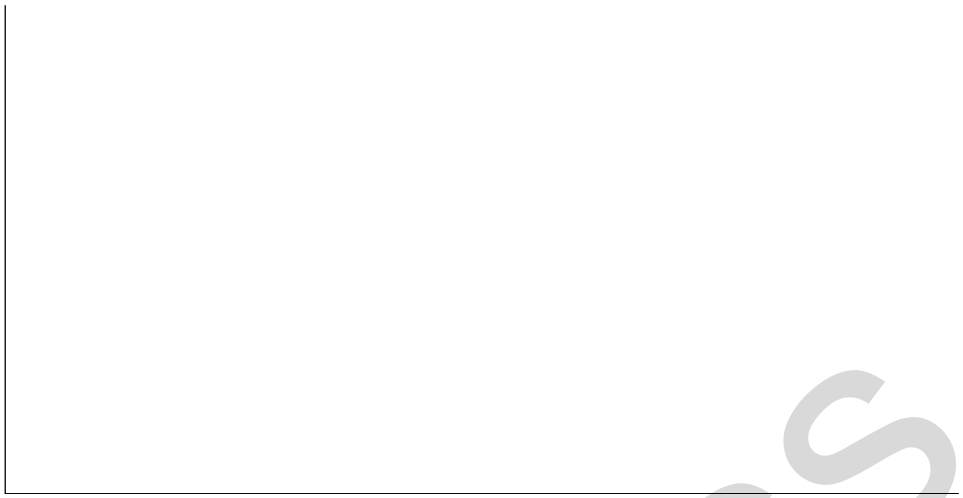
U_{PAH} = excreted amount =

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 $\mathbf{C_{PAH}}$ as the function of $\mathbf{P_{PAH}}$.

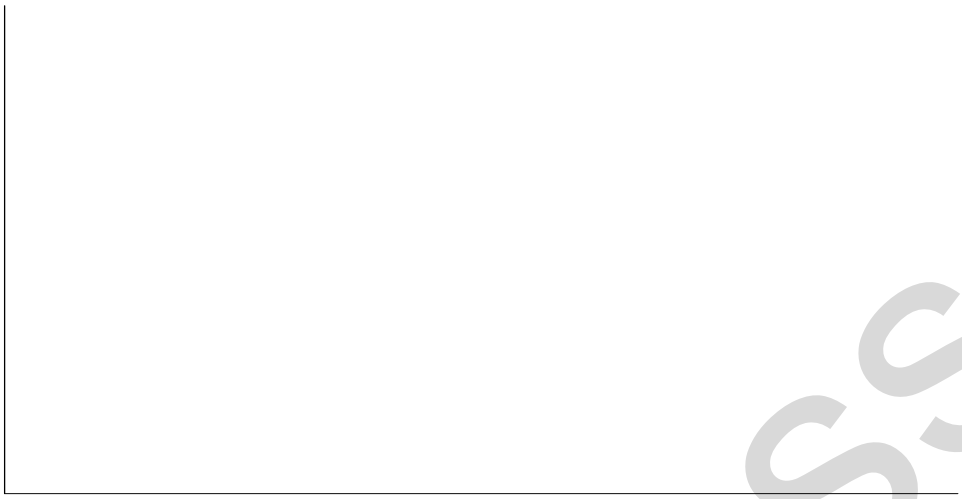


Plot $\mathbf{E_{PAH}}$ as the function of $\mathbf{P_{PAH}}$.

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

8.3. 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 Tm_G 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 (Tm_G) =

Decrease the reabsorption capacity and recalculate the value of Tm_G 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 =

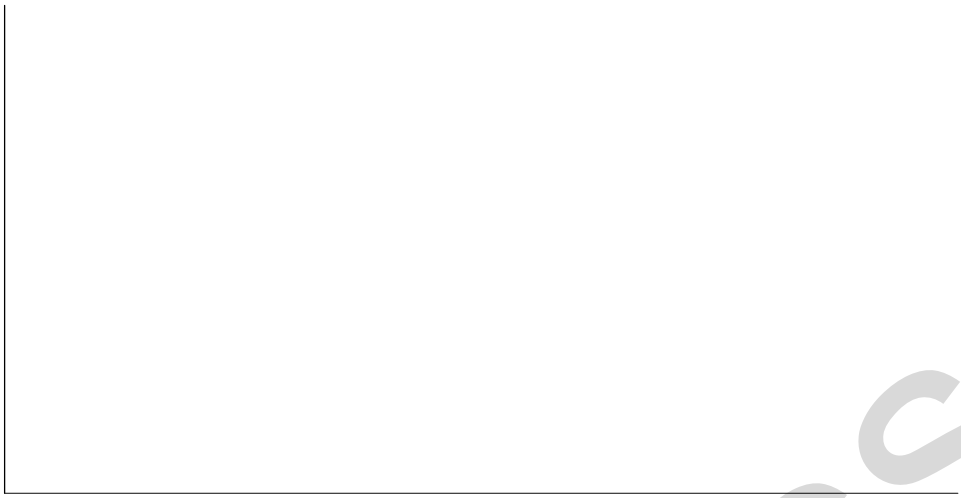
reabsorbed amount (Tm_G) =

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 $\mathbf{C_G}$ as the function of $\mathbf{P_G}$.



Plot $\mathbf{E_G}$ as the function of $\mathbf{P_G}$.

What is the theoretical maximum of $\mathbf{C_G}$ and $\mathbf{E_G}$?

The student was present:

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

The lab is completed:

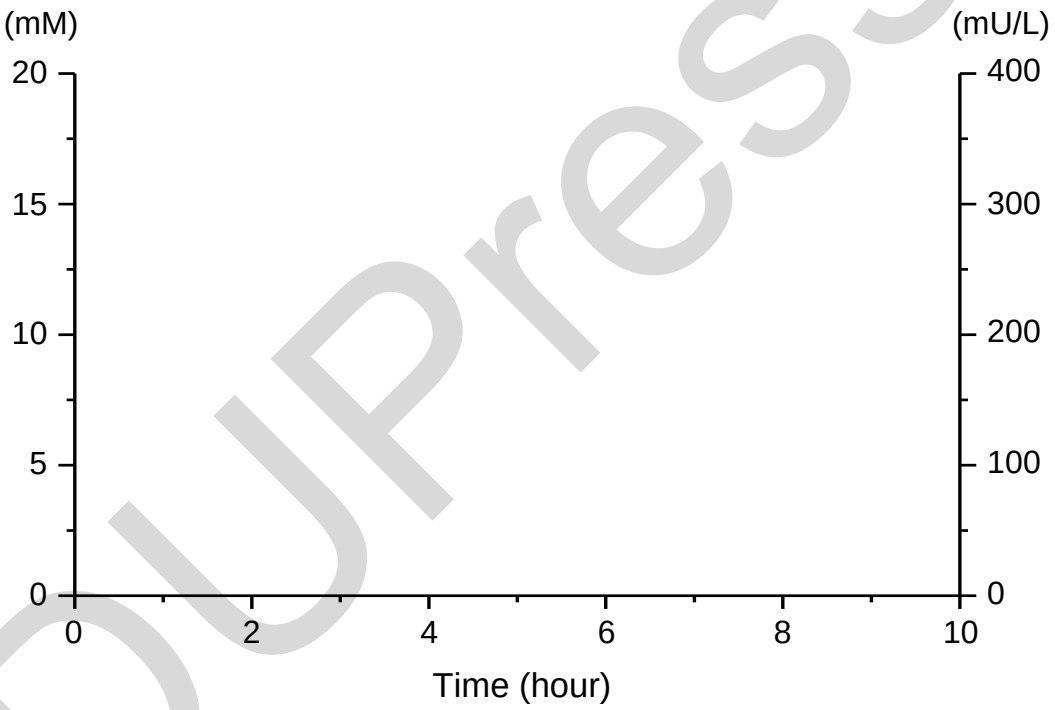
…….. .....
date signature of lab teacher

TOPIC SHEET N^{o} 9

COMPUTER SIMULATION OF THE GLUCOSE TOLERANCE TEST

9.1. 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.

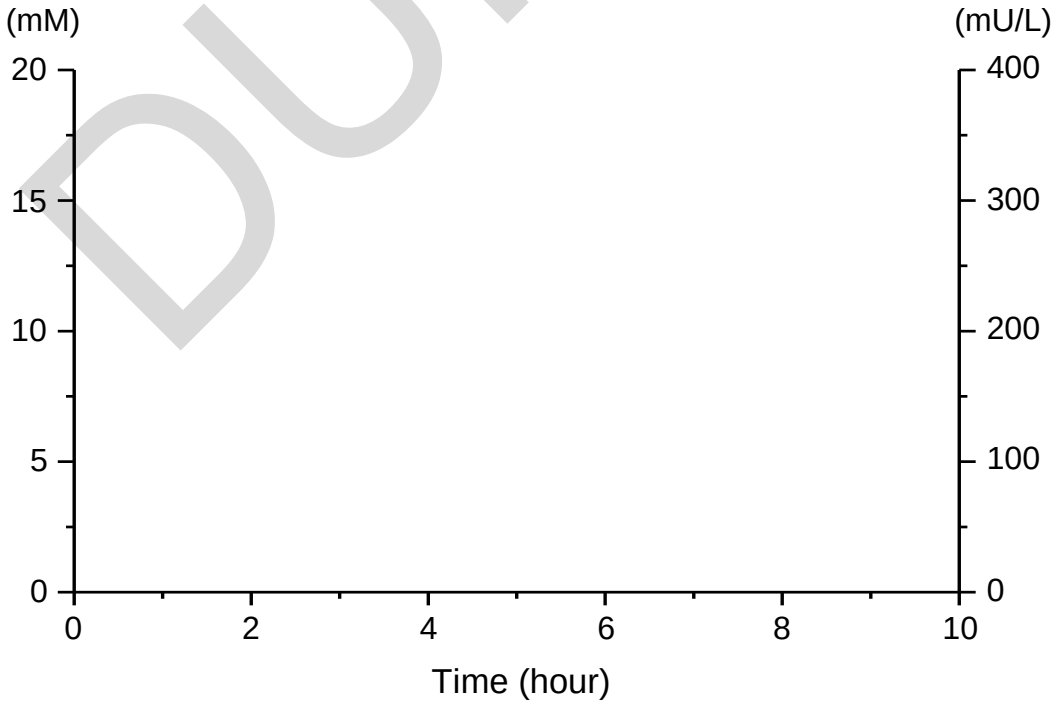


Fill out the table below using the data shown on the screen.

		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.

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

9.2.1. 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.

9.2.2. 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.

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

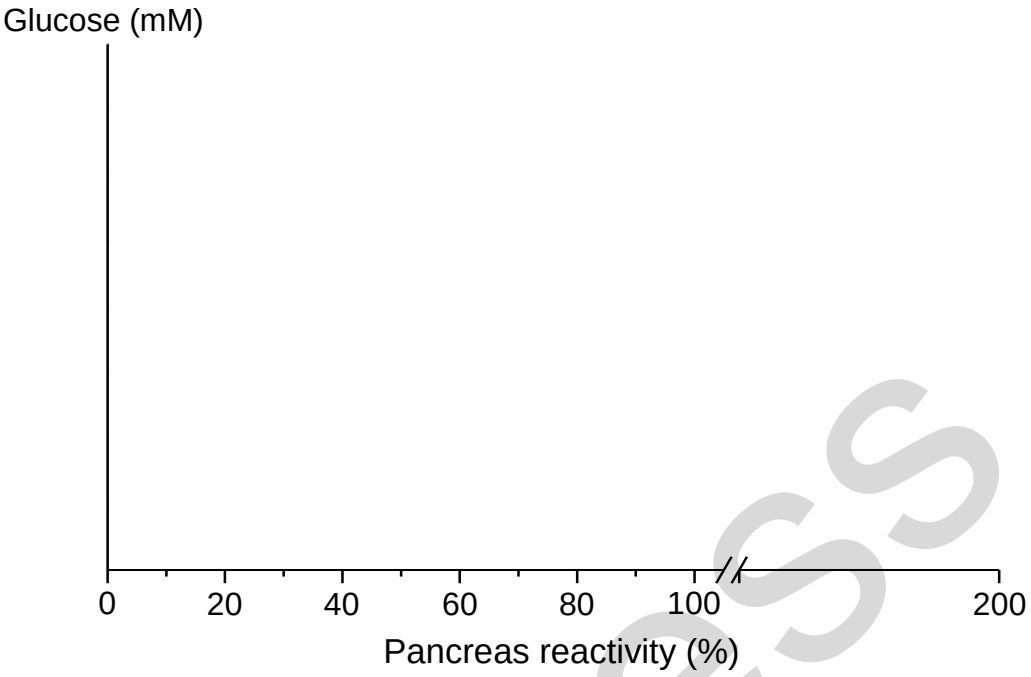
Select the DATA SETTING menu and change the relative reactivity of the pancreas to for instance 20, 50, 200 and 500% (pancreas reactivity should be at least 9% and cannot exceed 600%). Evaluate the changes in a few words by comparing the observed effects to the normal situation.

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

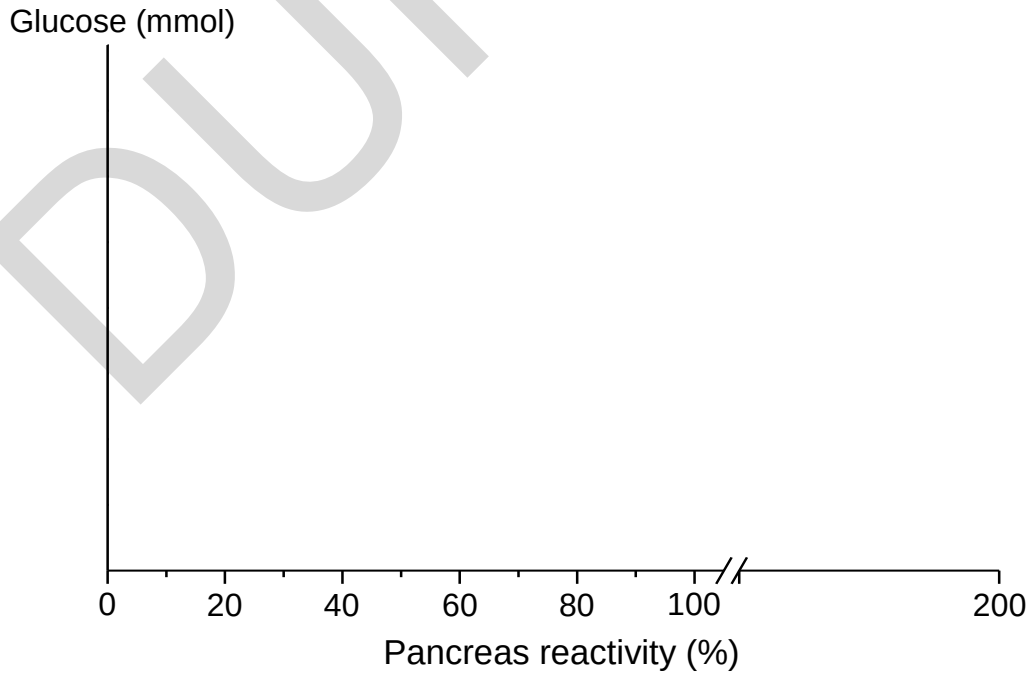
Fill out the table below by modifying the relative reactivity of the pancreas in the DATA SETTING menu. Use the double glucose load (Straub-Traugott mode)!

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?

The student was present:

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

The lab is completed:

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

TOPIC SHEET N° 10

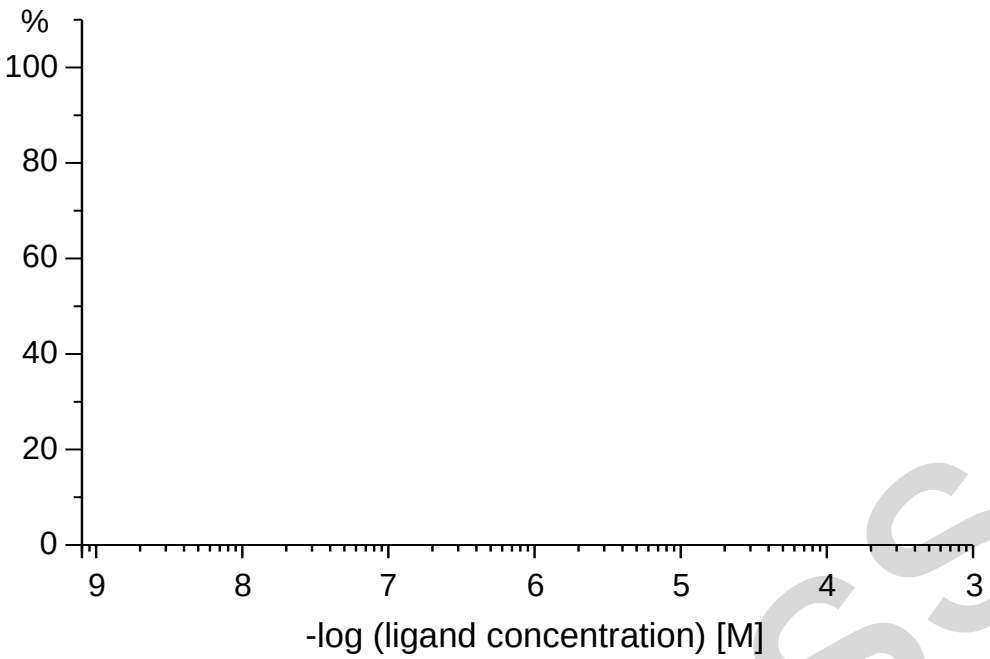
COMPUTER SIMULATION OF THE HUMORAL REGULATION OF THE INTESTINAL SMOOTH MUSCLE

10.1. 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.

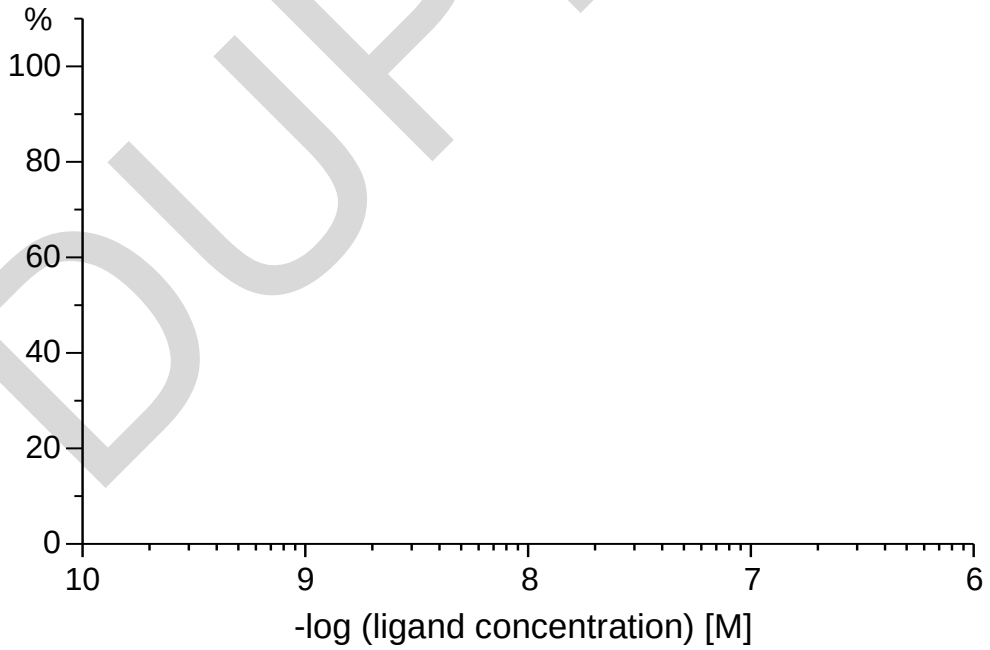
How do you explain the effect of acetylcholine? What kind of receptor is involved in the action of acetylcholine, and what second messenger pathway it is linked to?

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?

10.2. 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		

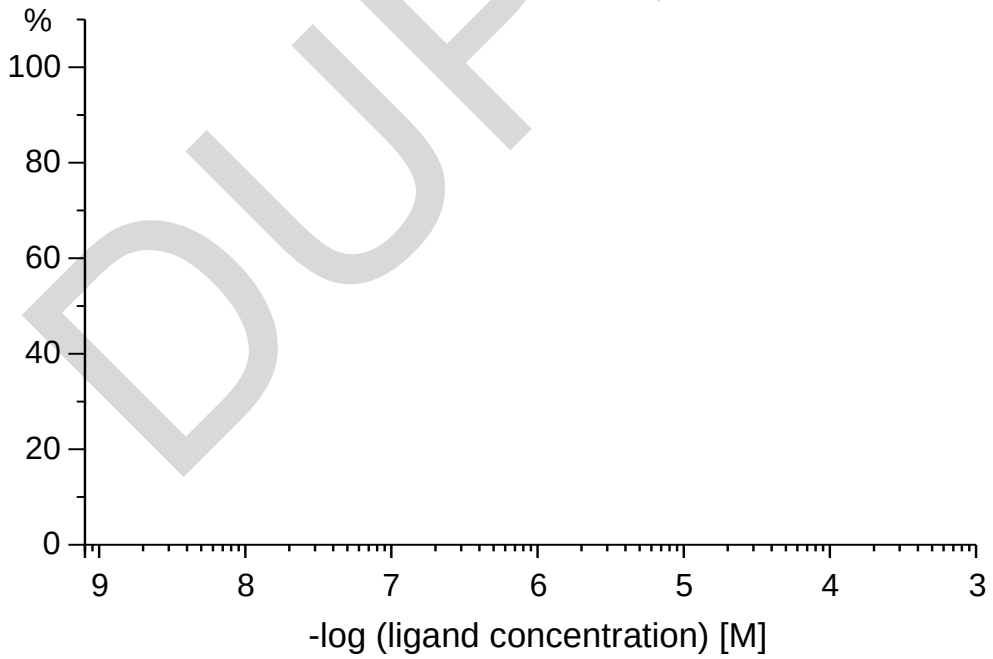


What is the half inhibitory concentration (IC_{50}) of atropine? Compare the affinities of the receptor for acetylcholine and atropine.

10.3. 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				

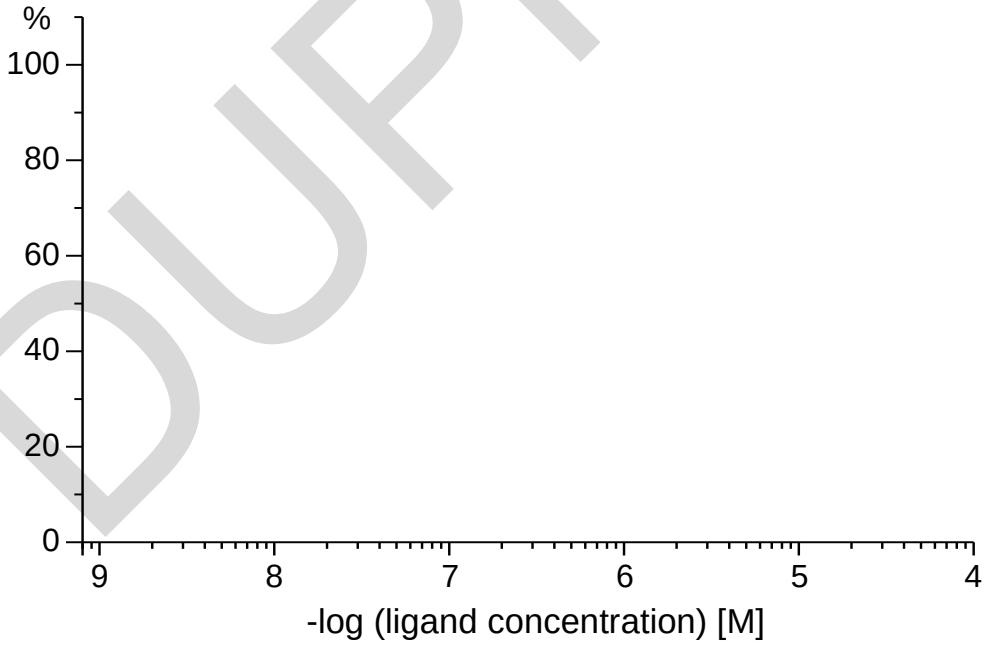


How do you explain the effect of physostigmine?

10.4. 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				

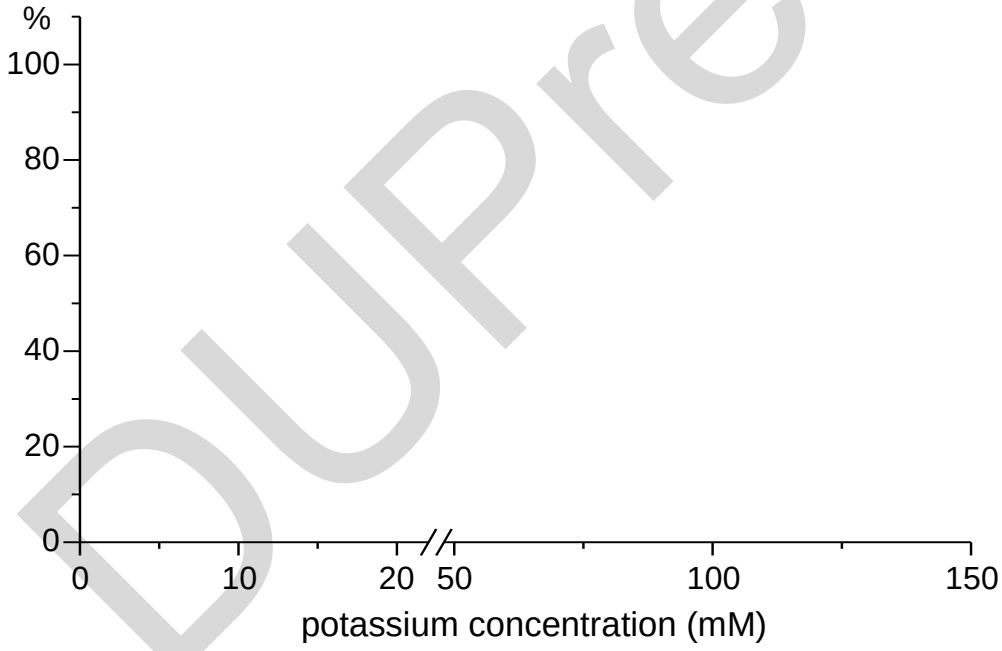


How do you explain the effect of histamine?

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

10.5. 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?

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

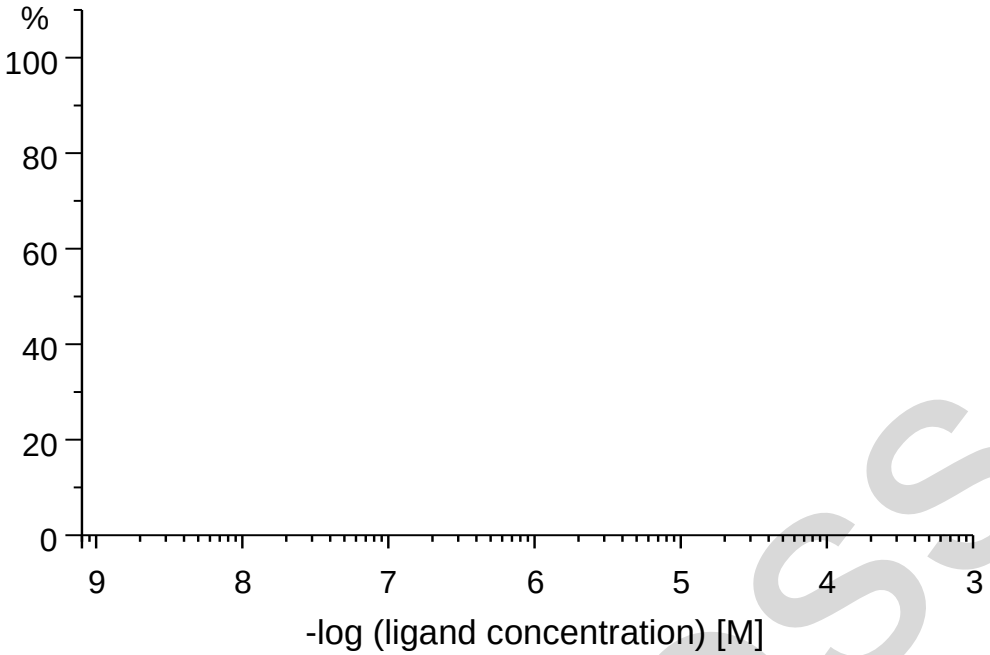
Working hypothesis:

Effects:

Results:

Ligand concentration (µmol/L)	Change in 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

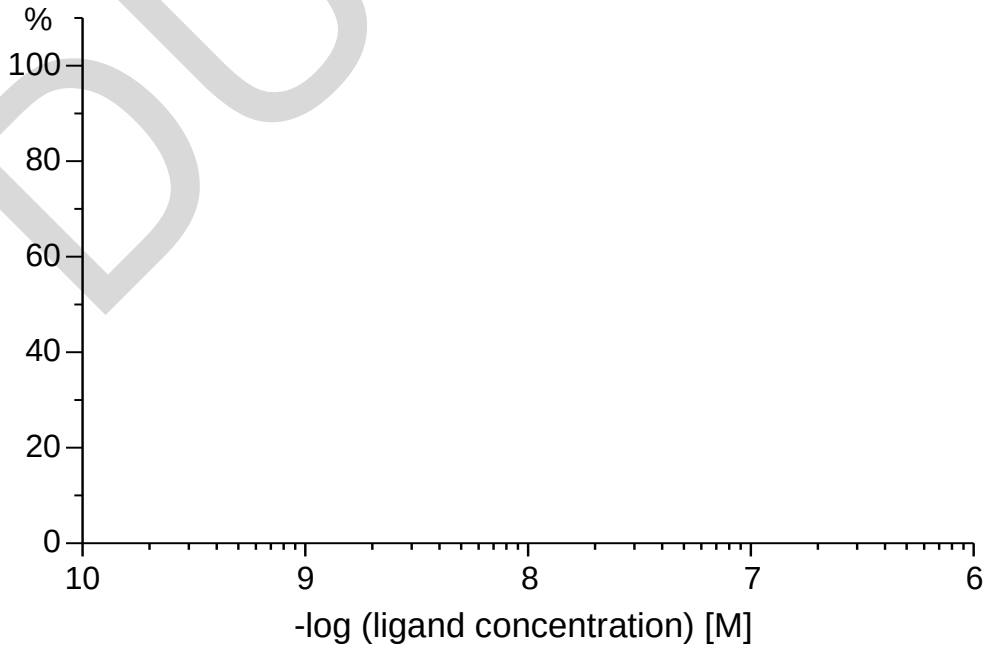
TOPIC SHEET N° 11

INVESTIGATION OF THE ENDOTHELIAL FUNCTION ON ISOLATED ARTERIAL RING

11.1. 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}).

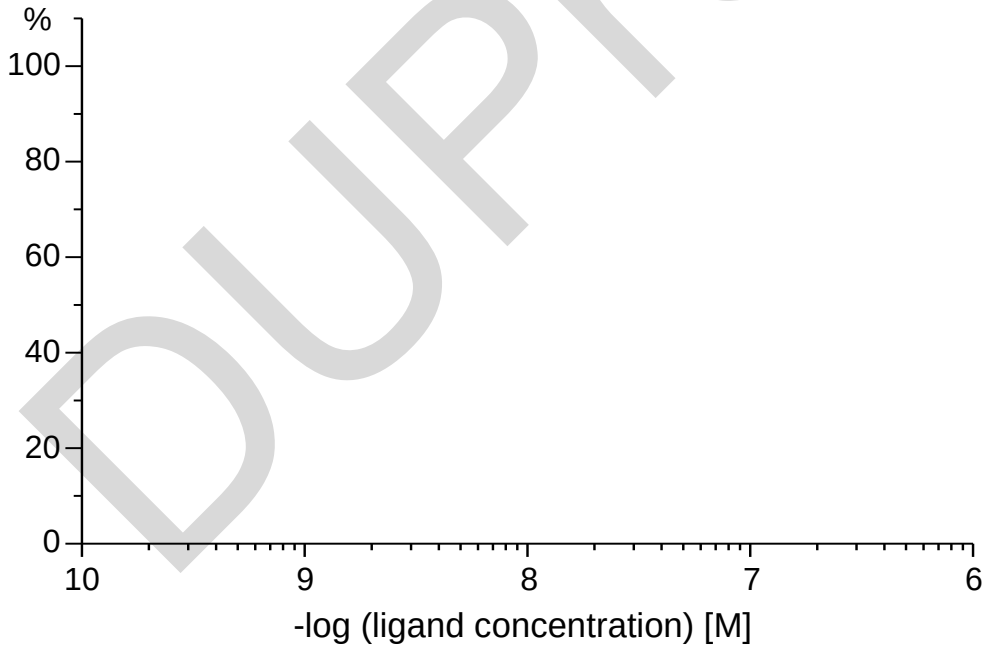
Norepinephrine concentration (mol/L)	**With intact endothelium**		**Without endothelium**	
	Measured value	Normalized to the measured maximum	Measured value	Normalized to the measured maximum
5×10^{-10}				
1×10^{-9}				
5×10^{-9}				
1×10^{-8}				
5×10^{-8}				
1×10^{-7}				
5×10^{-7}				
1×10^{-6}				



11.2. 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)

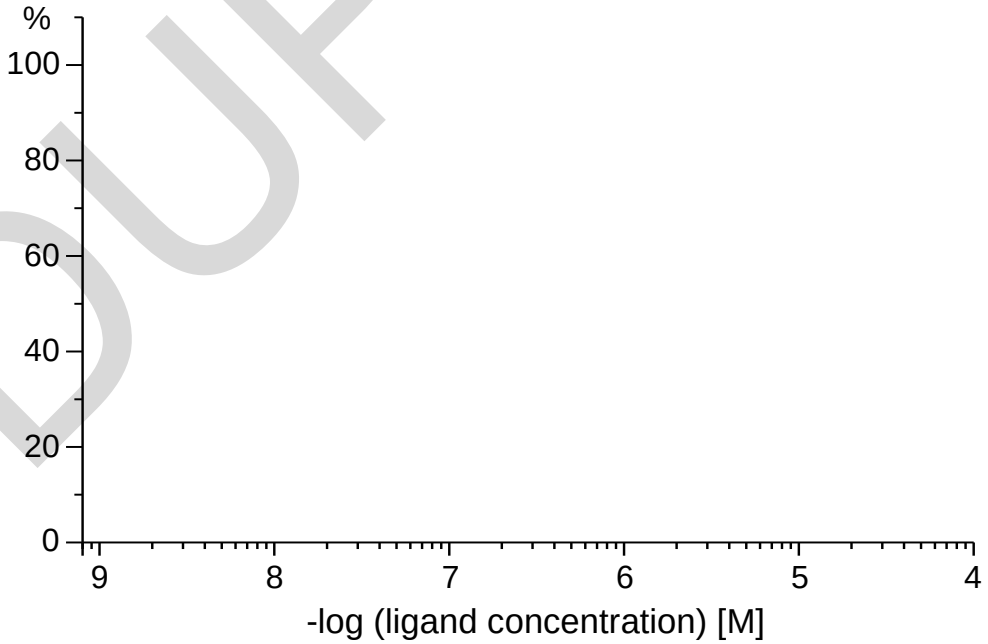
Norepinephrine concentration (mol/L)	With intact endothelium in the presence of **L-NMMA**		Without endothelium in the presence of **L-NMMA**	
	Measured value	Normalized to the measured maximum	Measured value	Normalized to the measured maximum
5×10^{-10}				
1×10^{-9}				
5×10^{-9}				
1×10^{-8}				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

11.3. 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.

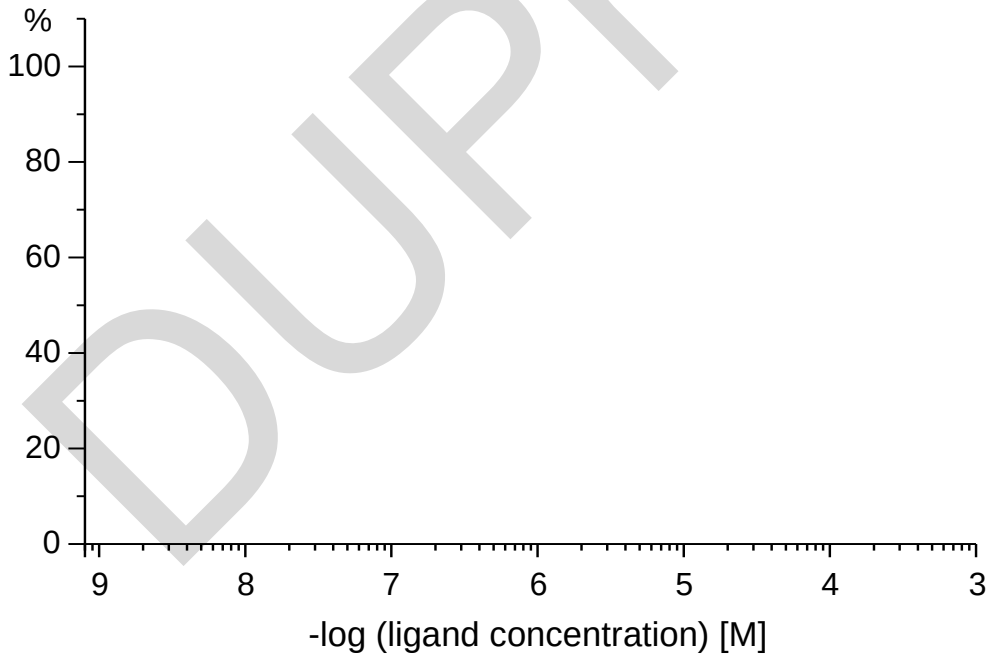
Acetylcholine concentration (mol/L)	With intact endothelium in the presence of **norepinephrine**		Without endothelium	
	Measured value	Normalized to the measured maximum	Measured value	Normalized to the measured maximum
0				
1 x 10^{-9}				
5 x 10^{-9}				
1 x 10^{-8}				
5 x 10^{-8}				
1 x 10^{-7}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

11.4. 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 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.

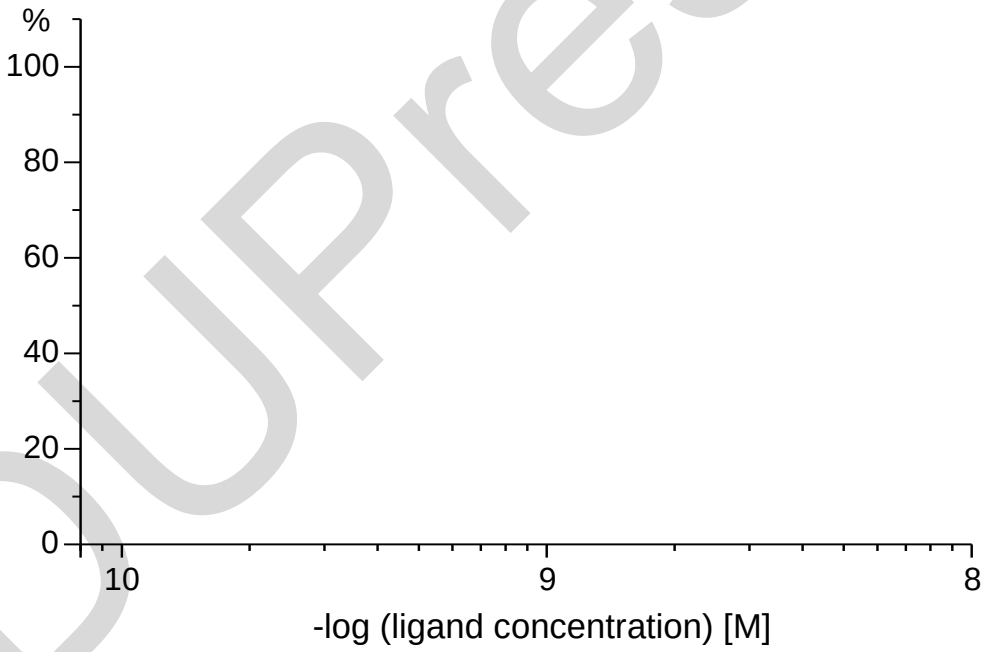
Acetylcholine concentration (mol/L)	Antagonist concentration		Antagonist concentration	
	Measured value	Normalized to the measured maximum	Measured value	Normalized to the measured maximum
0				



How do you explain the results?

11.5. 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?

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

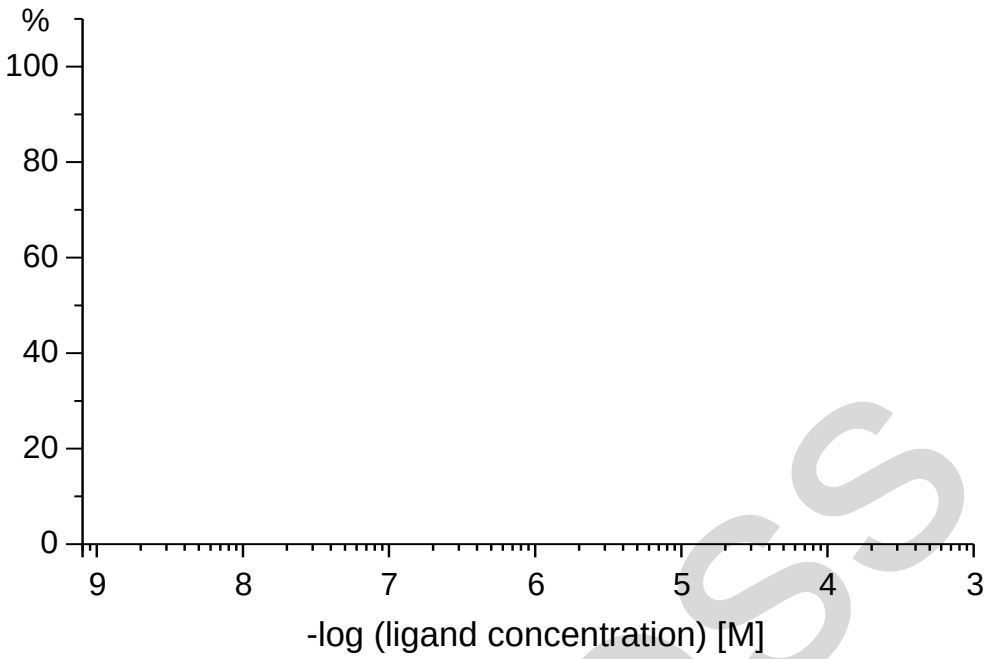
Working hypothesis:

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° 12

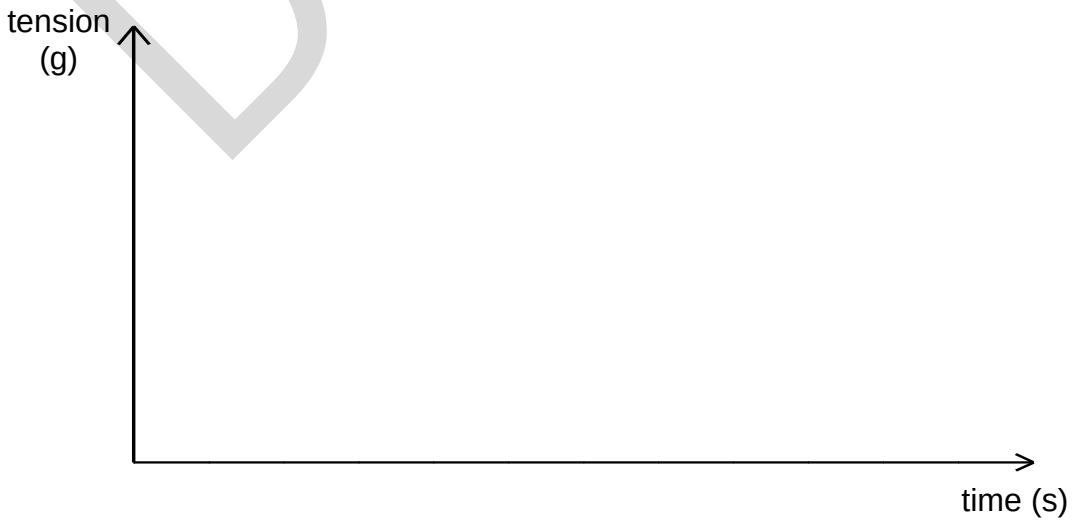
COMPUTER SIMULATION OF THE SKELETAL MUSCLE FUNCTION

12.1. Stimulus-dependent force generation

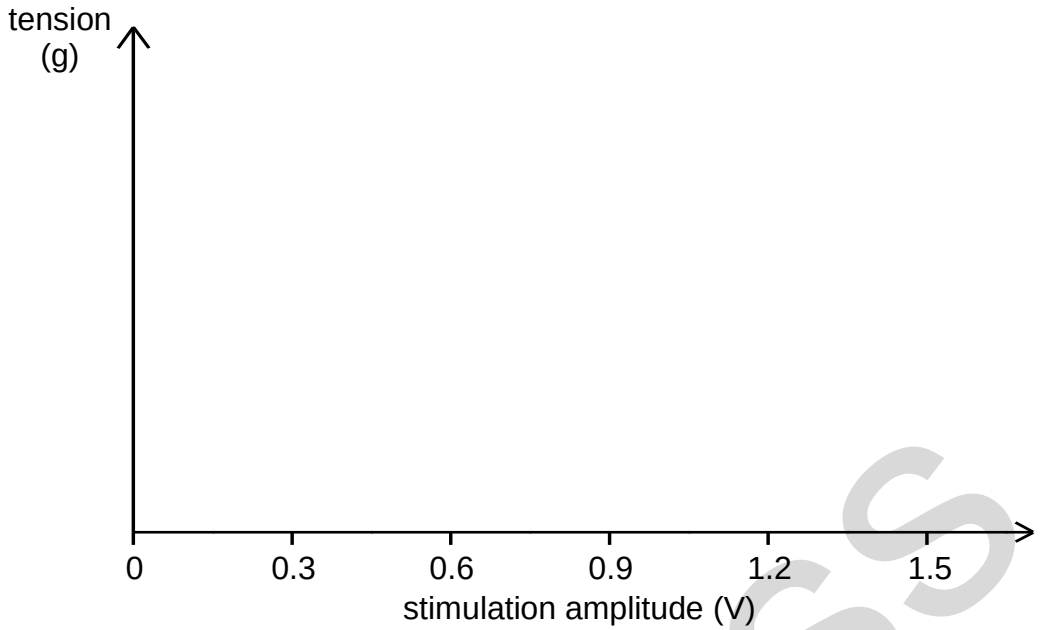
Choose the appropriate menu point in the "***Skeletal Muscle Function***" simulation program and examine the skeletal muscle tension using stimulation amplitude between 0 and 1.6 V!

stimulation amplitude (V)		tension (g)
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?

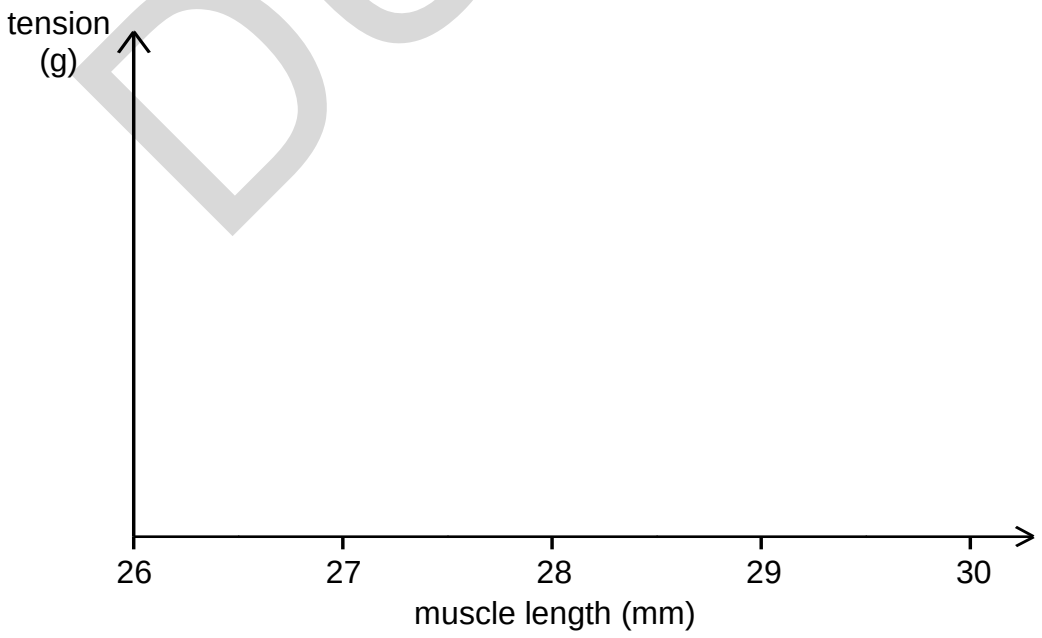
How do you explain the increased tension occurring when the amplitude of the stimulation is increased?

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

12.2. 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?

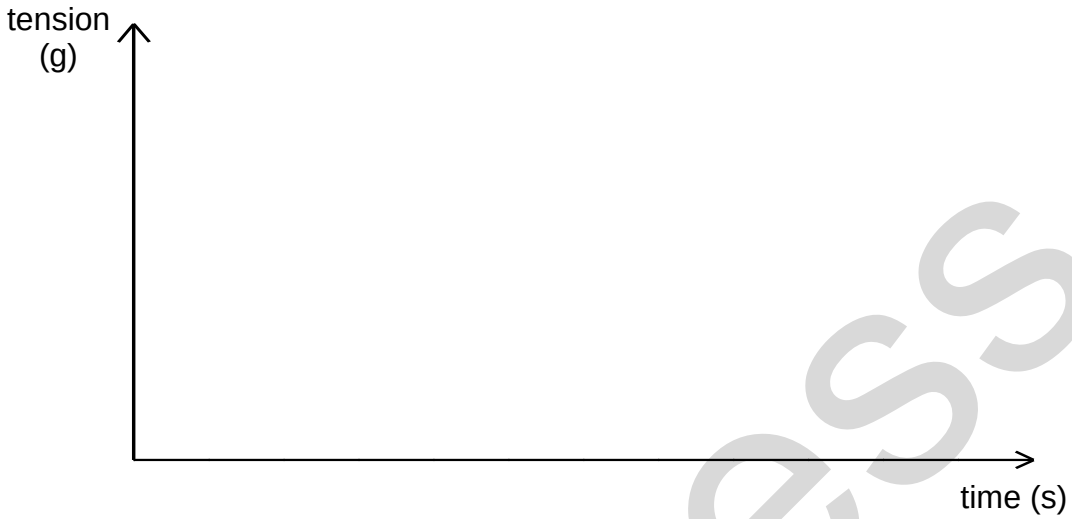
How does the muscle elongation affect the sarcomere length?

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

12.3. 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 relates 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 sarcoplasmatic 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

CONTENTS