Comenius University in Bratislava, Faculty of Medicine
Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry

LABORATORY PROTOCOL GM-WS - 4th seminar

Aerobic and anaerobic oxidation of glucose in brain tissue

Name, group:	Date:

Procedure:

Brain tissue is washed in cooled medium and homogenized in Krebs-Ringer incubation medium. **1 ml of** homogenate contains **200 mg of tissue, which is a source** of enzymes used for demonstration of glucose oxidation and measurement of glycolysis products under aerobic and anaerobic conditions. Specifically we measure:

1. amount of consumed glucose
2. amount of produced lactate

Determination of amount of glucose using Bio-La-test Oxochromglucose:
Glucose reacts with oxygen in a reaction catalyzed by *glucose oxidase*. Products of this reaction is gluconate and hydrogen peroxide, which reacts further with 3-methylphenol and 4-aminophenazone in a reaction catalyzed by *peroxidase*. The product of second reaction is a red-colored complex and the intensity of the color is measured spectrophotometrically.

Detemination of amount of produced lactate using ferriphenantroline:
Lactate produced from glucose under aerobic and anaerobic conditions is determined using lactate dehydrogenase, which converts it to pyruvate while the hydrogen atoms are transferred to NAD^+ to form $NADH+H^+$. Reduced coenzyme reacts with ferriphenantroline (Fe^{3+}-phenantroline), which is reduced to red-colored ferrophenantroline (Fe^{2+}-phenantroline). Intensity of the color can be measured spectrophotometrically.

(This part has been prepared by the assistant)		conditions	
		aerobic	anaerobic
Warburg vessel			
main space	postnuclear supernatant of 20% homogenate	1.0 ml	1.0 ml
	incubation medium	0.9 ml	0.9 ml
side arm	glucose 4.5 $mmol.l^{-1}$	0.6 ml	0.6 ml
incubated in atmosphere containing		O_2	N_2
After closing vessel we pour contents of side arm into the main space and the reaction starts from that moment on.			
We let the solution in Warburg stand for 30 minutes at laboratory temperature.			
stopping reaction using 30% TCA		0.5 ml	0.5 ml
After stopping the reaction we get 3 ml of supernatants which we use for measuring glucose (Glc) left in the vessel and produced lactate (Lac).			

Determination of remaining glucose:

sample	**Glc O_2**	**Glc N_2**	**reference sample**
glucose reagent	1.0 ml	1.0 ml	1.0 ml
supernatant (O_2)	0.1 ml	---	---
supernatant (N_2)	---	0.1 ml	---
water (by pipette)	---	---	0.1 ml
water (in dispenser)	0.9 ml	0.9 ml	0.9 ml
We let the samples stand for 15 minutes and measure absorbance at 500 nm.			

Determination of produced lactate

sample	**Lac O_2**	**Lac N_2**	**reference sample**
reaction mixture (in dispenser)	2.5 ml	2.5 ml	2.5 ml
supernatant (O_2)	0.1 ml	---	---
supernatant (N_2)	---	0.1 ml	---
water	---	---	0.1 ml
solution of LDH	0.1 ml	0.1 ml	0.1 ml
We let the samples stand for 20 minutes and measure absorbance at 510 nm.			

Calculation:

Amount of glucose in side arm before reaction (**amount of glc before the reaction starts**):
c (glc) = 4.5 mmol/l
V = 0.6 ml
n =

Glucose		**glucose left after reaction**		**consumption of glucose in reaction**		
	absorbance	µmol/0.1 ml	µmol/3.0 ml	µmol/3.0 ml	µmol/g	µmol/g/hour
Glc O_2						
Glc N_2						

ATP production from glucose:

Aerobic conditions: ……....µmol of glc/g/hour x 38 ATP = …….µmol of ATP /g/hour

Anaerobic conditions: ……....µmol of glc/g/hour x 2 ATP = ……..…….µmol of ATP /g/hour

Lactate		**amount of produced lactate**				
	absorbance	nmol/0.1 ml	nmol/3.0 ml	µmol/3.0 ml	µmol/g	µmol/g/hour
Lac O_2						
Lac N_2						

Conclusion:

Literature for next week:
- Glycogen metabolism (synthesis and degradation).
- Lippincott´s: Chapter 11 - Glycogen metabolism; formulas: Fig.11.3; 11.4; 11.7.
- Practical exercises in biochemistry (Asklepios, 1993) – Chapter 6 - Theoretical part.