

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

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LABORATORY PROTOCOL GM-WS - 5<sup>th</sup> seminar

**Influence of 48-hour starvation on blood glucose concentration  
and liver glycogen content in experimental animals**

Name, group:	Date:
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Principle:

Liver tissue is destructed using potassium hydroxide solution. KOH degrades proteins and monosaccharides, but it doesn't react with polysaccharides such as glycogen, which is stable in alkaline pH. Glycogen is precipitated in 96 % ethanol, then washed, suitably diluted and then hydrolyzed using H<sub>2</sub>SO<sub>4</sub>. The hydrolysate now contains glucose coming from glycogen and we can measure glucose concentration using oxochromglucose test.

Procedure:

sample	H <sub>1</sub>	S <sub>1</sub>	H <sub>2</sub>	S <sub>2</sub>	standard	reference
liver hydrolysate (well fed animal) (diluted 1:100)	0.1 ml	---	---	---	---	---
serum (well fed animal) (diluted 1:10)	---	0.1 ml	---	---	---	---
liver hydrolysate (starving animal) (non-diluted)	---	---	0.1 ml	---	---	---
serum (starving animal) (diluted 1:10)	---	---	---	0.1 ml	---	---
glucose standard (0.5 mmol/l)	---	---	---	---	0.1 ml	---
water (using pipette)	---	---	---	---	---	0.1 ml
water (in dispenser)	0.9 ml	0.9 ml	0.9 ml	0.9 ml	0.9 ml	0.9 ml
glucose reagent	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml

We let the samples stand for 10 minutes and measure absorbance at 500 nm.

Calculation:

$$c(\text{sample}) = A(\text{sample}) / A(\text{standard}) \times c(\text{standard})$$

Calculation of glycogen content:

To make 5 ml of hydrolysate 400 mg of liver tissue was used.  
Molecular weight of glucose is 180 g/mol.

400 mg of tissue..... in 5 ml of hydrolysate  
x mg of tissue..... in 1000 ml of hydrolysate

x =

1 mol of glucose..... 180 g  
 1 mmol of glucose..... 180 mg = 0.18 g

sample	well-fed animal		starving animal		standard
	H <sub>1</sub>	S <sub>1</sub>	H <sub>2</sub>	S <sub>2</sub>	
absorbance					
glucose concentration in mmol/l					0.5
correction for dilution					---
mmol of glucose / 100 g of liver tissue		---		---	---
grams of glucose / 100 g of liver tissue = glycogen content in %		---		---	---

Reference values:

Glucose in blood serum: **3.3 – 5.6 mmol/l.**

Liver glycogen content in well fed animal: **approx. 4-5%, at most 10% of liver weight.**

Conclusion:

**Literature for next week:**

Endocrine control of glucose metabolism and its plasma level.

- Practical exercises in biochemistry (Asklepios, 1993) – Chapter 7 - Theoretical part
- Lippincott's: Chapter 23 - Metabolic effects of insulin and glucagon.