1. ZOLLINGER - ELLISON SYNDROME

Zollinger Ellison syndrome is characterized by gastric hypersecretion, ulceration (ulceration) and gastrin-producing tumor. Gastrin is a peptide hormone synthesized by the G-cells of the stomach (and δ-cells of the pancreas) and has a stimulating effect on the production of hydrochloric acid. Gastrinoma often occurs in the pancreas. It is a malignant tumor and has metastatic potential. Most patients suffering from gastric ulcers and they are refractory to treatment. Diagnosis is based on anamnestic data, laboratory of gastrin levels (<95 pmol/L) and the investigation of gastric acid secretion.

Casuistics:
32 year old man comes to the clinic due to the chronic diarrhea. Recently patient experiences black stools.
He has been treated with bisoprolol 5 mg daily due to high blood pressure for several years.
Six months ago, he completed treatment for Helicobacter pylori eradication due to the duodenal ulcer. Sometimes he suffers burning feelings in the stomach area and overall discomfort. Recently, he feels tired, and he observed weight loss (about 8 kg in 2 months).
He is not a smoker, occasionally alcohol drinker (red wine). He is allergic to ampicilin. He works as bank clerk.

Clinical findings:
Height 178 cm, weight 65 kg, BP 145/80, RR 16/min, T=36,8ºC
Patient is conscious, orientated, actively cooperating. Skin is pale. Respiration alveolar, without side-phenomena. Cardiac action regular, 66 per minute, no murmur. Normal abdomen.

After the first sampling, the patient receives dose of i.v. pentagastrin (gastrin analogous) and the gastric secretion samples are taken again in 60 min time course. Samples are taken every 15 minutes - 4 times all together (MAO – maximal acid output). On the basis of the measured amounts of hydrochloric acid the level of hypersecretion can be estimated.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>58 g/ L</td>
</tr>
<tr>
<td>S-Na⁺</td>
<td>138 mmol/L</td>
</tr>
<tr>
<td>S-K⁺</td>
<td>4,2 mmol/L</td>
</tr>
<tr>
<td>S-Ca</td>
<td>2,75 mmol/L</td>
</tr>
<tr>
<td>S-Cl⁻</td>
<td>90 mmol/L</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>30 mmol/L</td>
</tr>
<tr>
<td>gastrin</td>
<td>480 pmol/ L</td>
</tr>
</tbody>
</table>

Physiological values: BAO: 3-5 mmol/h; MAO: 15-20 mmol/h

Our laboratory has received 5 samples of one patient. The first one is labelled BAO (see above). Four samples with labels MAO 1 – MAO 4 do represent the amount of secret taken in 15 minutes interval. Your task is to analyze the samples and estimate the level of HCl secretion disorder.

2. INSTRUCTIONS FOR ANALYSIS

2.1. Standardization of sodium hydroxide solution with oxalic acid

Sodium hydroxide is water soluble hygroscopic compound. Solutions prepared by dissolving of NaOH in water are not exactly defined, because NaOH is unstable. NaOH reacts with atmospheric water and carbon dioxide. It is necessary to find exact concentration by standardization. For alcalimetric titrations we use oxalic acid dihydrate as primary standard. On your lab desk you can find prepared oxalic acid solution with precise defined concentration.

Sodium hydroxide is titrated with oxalic acid solution as follows:

\[
\text{HOOC-COOH} + 2 \text{NaOH} \rightarrow \text{NaOOC-COONa} + 2 \text{H}_2\text{O}
\]

The endpoint is visualized with acid-base indicator - Tashiro (mixed indicator contains methyl red and methylene blue). Colour change is from violet in acid to green in basic solution (Fig. 2).

Oxalic acid is moderately strong acid. For correct determination of equivalence point, add just before the point calcium chloride solution.

Calcium chloride displaces hydrogen ions in the molecule of oxalic acid. This will convert titration to the titration of strong acid HCl with NaOH with a sharp colour change.

Procedure:
To make the NaOH solution, weigh 2.20 g of the solid sodium hydroxide. Add solid NaOH to approximately 50 mL of water (use beaker). Mix the solution with glass stirrer gently until NaOH is dissolved. Then adjust the volume in volumetric flask to 250 mL. Mix the solution well by turning upside down.

1. Rinse a burette with 10 mL of prepared sodium hydroxide solution. Discard the rinse solution. Repeat the rinsing on more time. Adjust the volume to the zero mark.

2. Measure 10 mL of oxalix acid standard solution to a 250 mL titration flask. Dilute with 30 mL of distilled water. Add 2 drops of Tashiro indicator solution. Concentration of standard oxallic acid solution is 0.1000 mol/L.
3. Titrate the sample to the grey colour. Then add 10 mL of 20 % calcium chloride solution. Mix it well and rinse the inner walls with distilled water. Carefully titrate to green colour (1-2 drops).

4. Record a volume of NaOH used. Repeat the titration procedure, above, 2 more times. From average used volume calculate the concentration of NaOH solution.

2.2. The BAO and MAO estimation

On every desk there are samples of gastric secretion taken by nasogastric tube. The samples are marked BAO and MAO 1 – MAO 4. Volumes are in the table:

<table>
<thead>
<tr>
<th></th>
<th>BAO</th>
<th>MAO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>150 mL</td>
<td>150 mL</td>
</tr>
<tr>
<td>BAO</td>
<td></td>
<td>MAO 1</td>
</tr>
<tr>
<td></td>
<td>150 mL</td>
<td></td>
</tr>
<tr>
<td>MAO 1</td>
<td></td>
<td>MAO 2</td>
</tr>
<tr>
<td></td>
<td>120 mL</td>
<td></td>
</tr>
<tr>
<td>MAO 2</td>
<td></td>
<td>MAO 3</td>
</tr>
<tr>
<td></td>
<td>80 mL</td>
<td></td>
</tr>
<tr>
<td>MAO 3</td>
<td></td>
<td>MAO 4</td>
</tr>
<tr>
<td></td>
<td>80 mL</td>
<td></td>
</tr>
<tr>
<td>MAO 4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BAO estimation:
On your desk there are samples of gastric secretion taken by nasogastric tube. Your task is to analyze and compute BAO. For titration use the earlier standardized NaOH solution.

1. Fill the burette with NaOH solution up to the marked level.

2. Into the rinsed titration flask add 10 mL of the gastric secretion sample.

3. Sample in the titration flask dilute by approximately 20 mL of distilled water and titrate using Tashiro indicator to the equivalence.

4. Estimation has to be carried out twice. Using the estimated NaOH consumption compute the HCl amount of substance, and subsequently BAO.

MAO estimation:
On your desk there are samples of gastric secretion labelled MAO 1- MAO 4.

1. MAO estimation carries out using identical way as in previous case (BAO). Each sample has to be analyzed twice. All together there will be carried out 8 estimations.

2. When finished, please, do not forget to rinse the burette by distilled water and make up the desk.
3. Results

3.1. Standardization of the NaOH solution

c(NaOH) = ……………………………

Calculation:

3.2. BAO a MAO estimation

A/ BAO calculation:

B/ MAO calculation:
Write your results into the following table:

<table>
<thead>
<tr>
<th>Sample</th>
<th>BA0</th>
<th>MAO 1</th>
<th>MAO 2</th>
<th>MAO 3</th>
<th>MAO 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (cm³)</td>
<td>150</td>
<td>150</td>
<td>120</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>NaOH usage (cm³)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average NaOH usage (cm³)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n(HCl) in analyzed sample volume (mmol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n(HCl) whole sample volume (mmol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Σn(HCl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( n = \text{amount of substance} \)

**Discussion**

1. How would you explain estimated patient’s BAO and MAO values?
2. Describe, how would change the experiment precision, in case of not using CaCl₂ in the titration.
3. Try to explain the occurrence of diarrhoea. Try to explain the cause.
4. Find the abnormal laboratory results and try to outline the reasons of theirs changes.
5. Why is ulcer dangerous and describe possible complications.

**Conclusion**
APPENDIX 1: MATERIAL SAFETY DATA SHEET

1. Hydrochloric acid

Risk phrases:
H314 Causes burns.
H335 Irritating to respiratory system.

Safety phrases:
H314 Causes burns.
P280 Wear suitable protective clothing, gloves and eye/face protection.

2. Sodium hydroxide

Risk phrases:
H314 Causes burns.

Safety phrases:
P280 Wear suitable protective clothing, gloves and eye/face protection.

3. Oxalic acid

Risk phrases:
H302/312 Harmful if swallowed or in contact with skin

Safety phrases:
P262 Do not get in eyes, on skin, or on clothing
P302/352 Wash with plenty of soap and water