2.3.9. Isolation and culture of normal and diabetic rat hepatocytes followed by determination the capacity of insulin metabolism in liver

2.3.9.1. Preparation

All buffers are freshly prepared using sterile technique.

1- Prepare Perfusion buffer I by adding the following to Hank's Balanced Salt Solution (HBSS, without Ca^{2+} and Mg^{2+}): Mg^{2+} ($MgCl_2$) to 0.9 mM, EDTA to 0.5 mM, and Tris base to 0.5 mM.

2- Prepare Perfusion buffer II by adding to HBSS (with Ca^{2+} and Mg^{2+}): Tris base to 0.5 mM.

3- Prepare Perfusion buffer II plus collagenaseII: Dissolve collagenase II (1000 U) with 300 ml Perfusion buffer II and keep the solution warm in water-bath before perfusion. This solution should be used within 30 min because the activity of collagenase II decreased with the time.

4- Prepare William's complete Medium: Add the following to Williams' Medium E: L-glutamine to 2 mM, fetal bovine serum (FBS); dexamethasone to 100 nM, penicillin to 100 IU/ml and streptomycin to 100 mg/ml.

5- These buffers should be warmed for 30 minutes in the water bath at 42 °C, an optimal temperature corresponding to an outlet temperature at the cannula of 37 °C.

2.3.9.2. Rat Perfusion for Liver Isolation

In situ liver perfusion technique as described above. After anesthesia of adult normal and diabetic rats and the surgery was performed. The perfusate tubing was connected