CHAPTER III

Materials & Methods

Chemical Substances

- Streptozotocin (STZ)
- Nicardipine
- Cilazapril
- Normal saline solution (NSS)
- Heparin
- Nembutal
- Perfusate solution

NaCL	118.0	mMol/L
KCL	4.7	"
$CaCL_2$	2.52	"
$MgSO_4$	1.66	"
$NaHCO_3$	24.88	"
KH_2PO_4	1.18	"
$C_6H_{12}O_6$	5.85	"

Bovine serum albumin 2 gm/100 ml

pH = 7.4

$O_2 : CO_2$ = 95% : 5%

Animal preparation :

Male Wistar Furth rats, weighing about 100-150 gms (aged 4-5 weeks) were used in this study (n=72).

All animals were fasted overnight before the diabetic induction using streptozotocin (STZ) (55 mg/kg BW, intravenous injection(I.V.)) The animals were separated into four groups

1. Control group (NSS) : The animals received an intravenous (I.V.) injection of normal saline solution (NSS) instead of STZ (n=18) .

2. Diabetic group (STZ) : The animals received a single I.V. injection of STZ (55 mg/kg BW). This group was treated daily with a NSS via oral feeding starting 1 day after the STZ injection until the day of performing the isolated heart experiment (n=18).

3. Diabetic with nicardipine treated group (STZ-N) : These animals received the STZ injection the same as the diabetic group. This group was treated with nicardipine (10 mg/kg BW/day) via oral feeding starting 1 day after the STZ injection until the day of performing the isolated heart experiment (n=18).

4. Diabetic with nicardipine combined with cilazapril treatment group (STZ-NC) : These animals received the STZ injection the same as the diabetic group. This group was treated with nicardipine (10 mg/kg BW/day) via oral feeding starting 1 day after the STZ injection until the day of performing the isolated heart experiment (n=18).

In each group, the cardiovascular parameters were accessed at 8, 16 and 20 weeks after the normal saline or STZ injection. These parameters are :

1. Systemic arterial pressure
2. Heart rate
3. Aortic flow rate
4. Coronary flow rate
5. Left ventricular contraction
6. Ratio of heart weight per 100 grams body weight

Method :

On the day of isolated heart experiment, the animal was weighed and then anesthetized by intraperitoneal injection (i.p.) of 30 mg/kg body weight of sodium pentobarbital. After tracheostomy, animal were ventilated with a small animal respirator (Harvard Rodent model 683). Blood pressure was measured by inserted catheter (PE 180) via common carotid artery and connected to the pressure transducer (Nikhon model TP-300T), then shown on the polygraph (Nikhon RM 6000). The chest was opened in order to remove the heart and the pericardial sac. Two vessels, the right subclavian artery, and the ascending aorta were then loosely ligated as shown in Figure 3.1.

Before the isolation of the heart, the values of aortic flow rate were measured by the flow probe (Nikhon model FE-020T) which placed around the ascending aorta. After these measurements, the right subclavian artery was done and 150 units of heparin was injected into the right atrium. The common-carotid-artery catheter was then connected to the perfusate system and the right atrium was then quickly cut open. The ligature on the ascending aorta was then tight directing the perfusate flow retrograde to the coronary circulation. The hearts then carefully removed from the animal. After the heart allowed to equilibrate for 15 minutes, the coronary flow rate was measured as the volume of fluid that flowed out from the cut right atrium per unit time (Figure 3.2). The left ventricular isotonic contraction was recorded through the wire hooked at the apex of left ventricle and connected to isotonic transducer (Figure 3.3). The patterns of contraction were recorded on the polygraph (Nikhon RM 6000).

At the end of each experiment, the hearts disconnected from perfusate system and weighted. The heart then soaked and preserved in 10% formalin solution for further pathological examination. The top and the apex of each heart were cut off with the size of 2 mm. thickness as shown in Figure 3.4, and then the hearts were horizontal cut equally into four pieces. Each piece was about 3-4 mm thick. The second piece which was the largest band was used for further morphological examinations as described as follows.

Morphological examinations

From the second piece which is the largest band, it was then further cut into 2 pieces. The first piece was processed by using Miller's elastic stain for morphological studies of the intramural coronary arteries. And the second piece was further stained with eosin and hematoxylin in order to assess the thickness of the ventricular wall.

The thickness of left ventricular wall (LV), right ventricular wall (RV) and interventricular septal wall (IVS) were measured randomly by the micrometer of light microscope with 40 x - objective. The measurement was performed randomly at five positions of each wall, as shown in figure 3.5. Mean and standard deviation (SD) of these five values of each wall were calculated and represented as the wall thickness of LV, RV and IVS.

Moreover, the thickeness of arterial wall was also measured using the second piece of each heart that was collected and stained with elastin. The arterial wall thickness was magnified by the light microscope with 400 x - objective and also videotape recorded for further measuring the area of wall thickness by using computer imaging analysis called Global-Lab Image software.

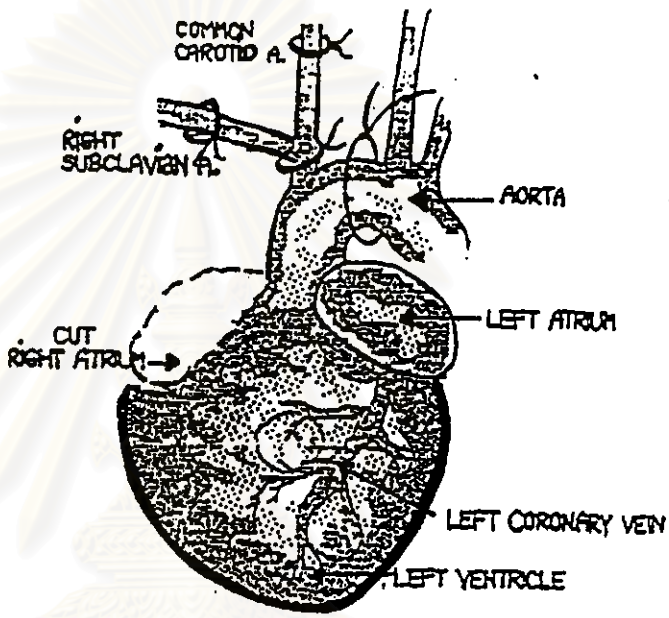

Figure 3.1 Cannulation procedure for perfusing the rat heart prior to isolation :

1. ligate the right subclavian artery
2. insert and secure catheter in common carotid artery
3. cut right atrium after beginning perfusion
4. ligate aorta immediately

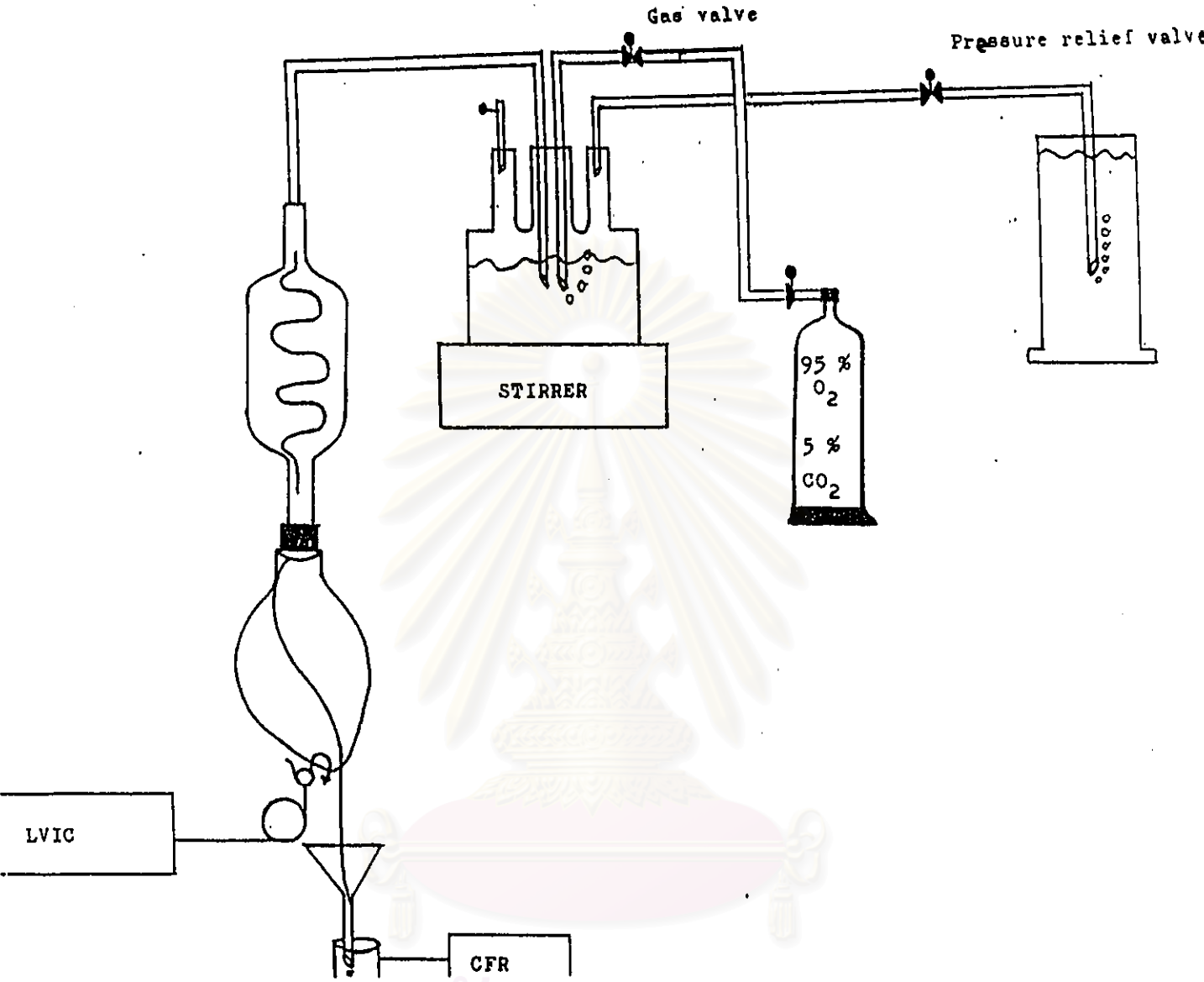

Figure 3.2 Experimental set - up for constant pressure perfusion of isolated rat heart.

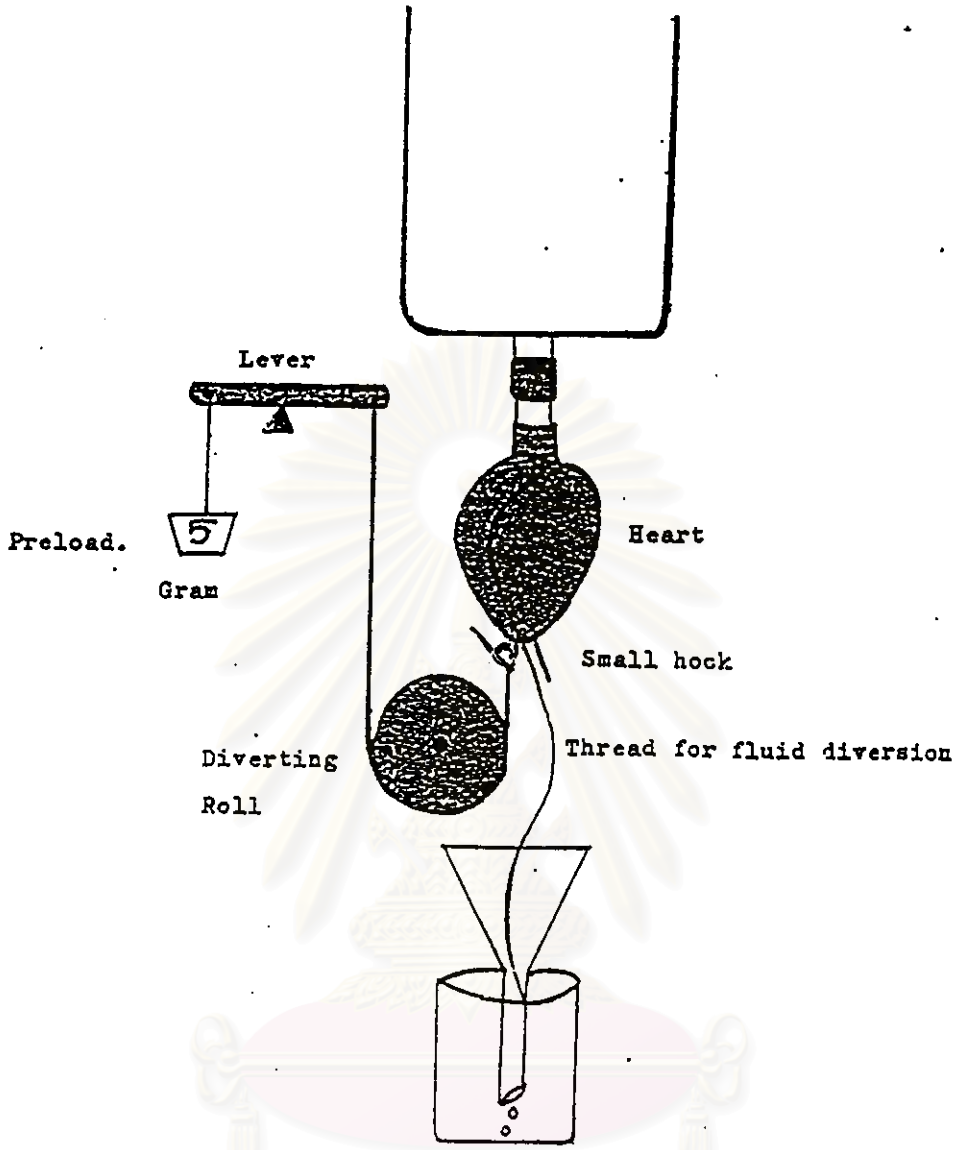

Figure 3.3 The force of contraction of each heart was measured with the preload of 5 gram.

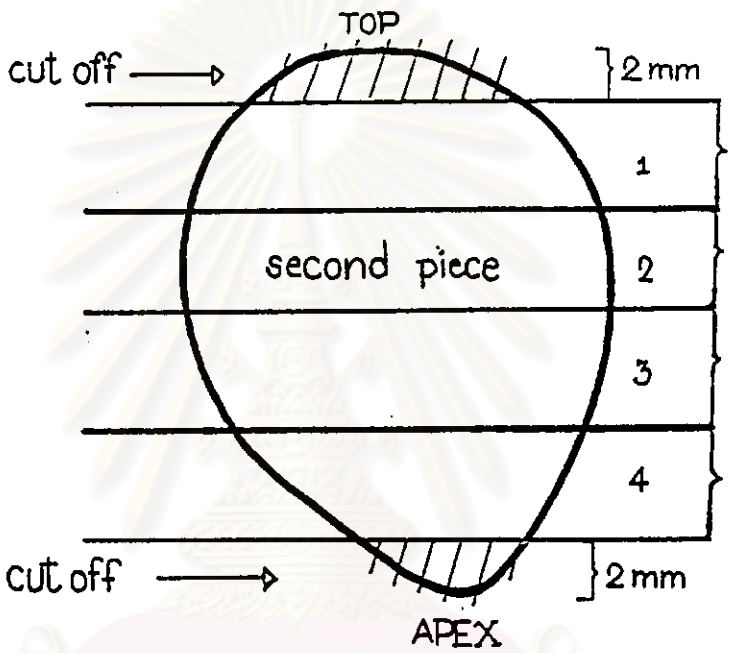

Figure 3.4 The heart was divided equally into four pieces. The thickness of each piece was about 3 - 4 mm. The second piece was then fixed by Eosin and Hematoxylin method for further morphological examinations.

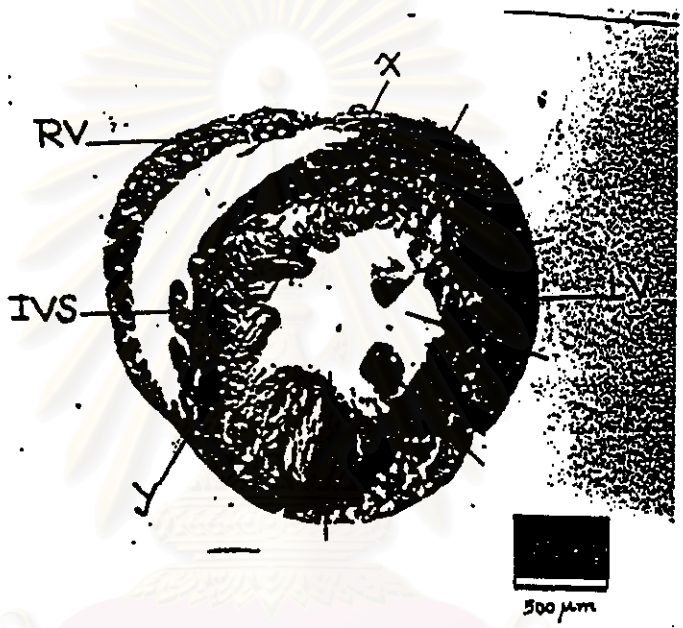

Figure 3.5 The example of five positions were randomly selected for measuring of wall thickness of left ventricle (LV).

The xy - line was located by connecting the points of RV and LV juctions. The walls of left ventricle and IVS were separated by this xy - line.