CHAPTER III

MATERIAL & METHOD

Chemical substances

Streptozotocin (STZ)

Cilazapril

Heparin

Nembutal

Perfusate solutions

	mMol/L
NaCl	118.00
KCl	4.70
$CaCl_2$	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 preparations

Male Wistar Furth rats weighing about 100-150 gm with age of 4-5 weeks were used in this study (N=45).

Animals were fasted overnight before the diabetic induction. The animals could be separated into three groups :

1) Control group (NSS) : the animals were received intraperitoneal (i.p.) injection of normal saline solution (N=15)

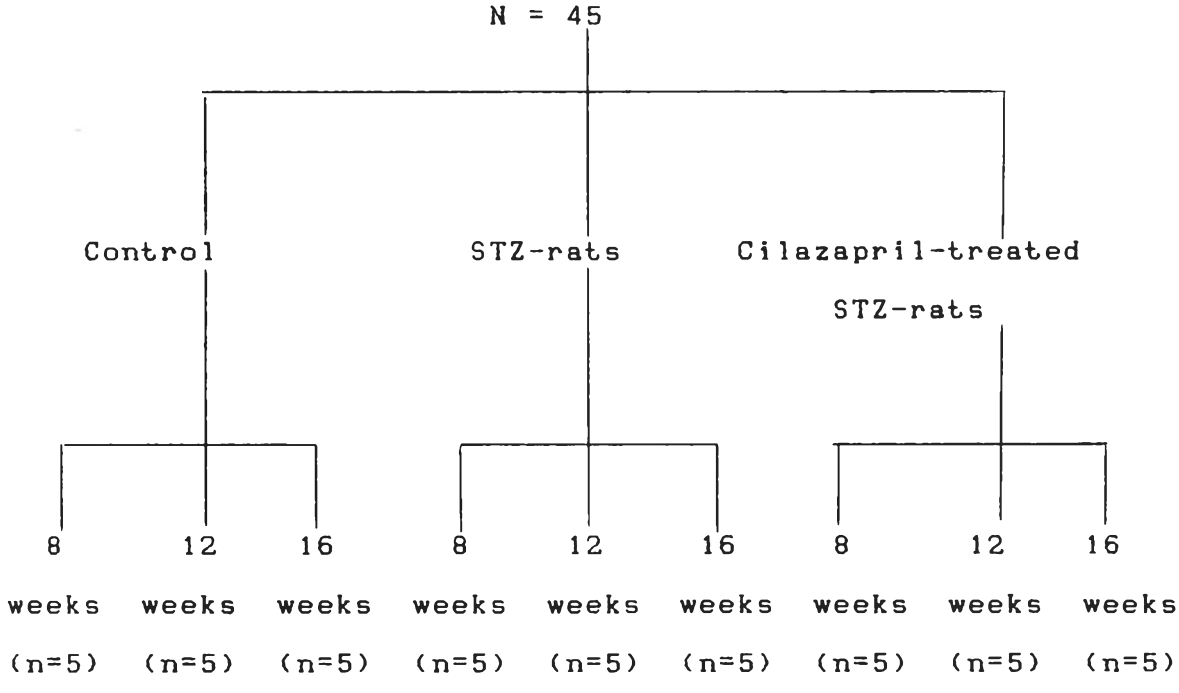
2) Diabetic group : the animals were received single i.p. injection of STZ (65 mg/kg body weight)

3) Diabetic with cilazapril treatment group

These animals were received the STZ injections the same as the diabetic group. This group was treated daily with cilazapril (10 mg/Kg body weight/day) via oral feeding starting 1 hour after the STZ injection until the day of performing isolated heart experiment.

Starting one week after STZ injection, weight and blood glucose of all rats were monitored. The hyperglycemic state was confirmed by polyuria, polyphagia, polydipsia and blood glucose concentration of 400 mg/dl or higher. The body weight of each rat was checked again at the day of performing experiment.

In each group, the animals were used in the performing of isolated heart experiment at 8, 12 and 16 weeks after the normal saline or STZ injections. Such these groups of five rats were refered as the three different aged groups as shown in the following diagram :



Methods

On the day of isolated heart experiment, the animal was weighing and then anesthetized by intraperitoneal injection (i.p.) of 30 mg/kg body weight of sodium pentobarbital. After tracheostomy, animals were ventilated witha small animal respirator (Harvard Rodent model 683). The chest was opened to expose the heart. The pericardial sac was carefully removed. Three vessels, the right subclavian artery, the innominate artery, and the ascending aorta were then loosely ligated. (Figure 3.1) The aortic flow rate was measured by the flow probe (Nikhon model FE-020T) placed on the ascending aorta.

Before the isolation of the heart, the values of aortic flow rate were measured by the flow probe which placed on the ascending aorta. The common carotid arterial pressure (CAP) was recorded via the catheter (PE 180) that

inserted into the common carotid artery by using pressure transducer (Nikhon model TP-300T) that connected to the polygraph (Nikhon RM 6000). After these measurements, the ligature of the right subclavian artery was tied 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 tied; directing the perfusate flow retrograde to the coronary circulation. The hearts were then carefully removed from the animals. After the hearts were allowed to equilibrate for 15 minutes, the coronary flow rate was measured as the volume of fluid that vent 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). With preloaded 5 grams, the patterns of contraction were recorded on the polygraph (Nikhon RM 6000).

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

Morphological examinations

The first piece of each heart were fixed with Elastic method for morphological studies of the intramural coronary arteries. The second piece of each heart that was collected by the method described in Figure 3.4 was further fixed with Eosin & Hematotoxylin. All specimens were obtained totally from three controls, three STZ-rats and three cilazapril-treated STZ-rats from each aged group (8, 12, 16 weeks). These specimens of the hearts were used to assess the thickness of ventricular wall and of coronary arteries 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 4x-objective. The measuring were performed randomly at five positions of each wall, as showed in Figure 3.5. Mean and SD of these five values of each wall were calculated and represented as the wall thickness of LV, RV and IVS

Note : The morphological examination of cross-section specimens of the hearts obtained in this investigation did not provide sufficient spatial resolution, especially of the coronary arterial wall compartment. Therefore, the wall thickness of coronary arteries could not be evaluated. The results of this section will be described as general observation of selected coronary arteries, and comparison between control and STZ-rats, and between STZ-rats and cilazapril-treated STZ-rats.

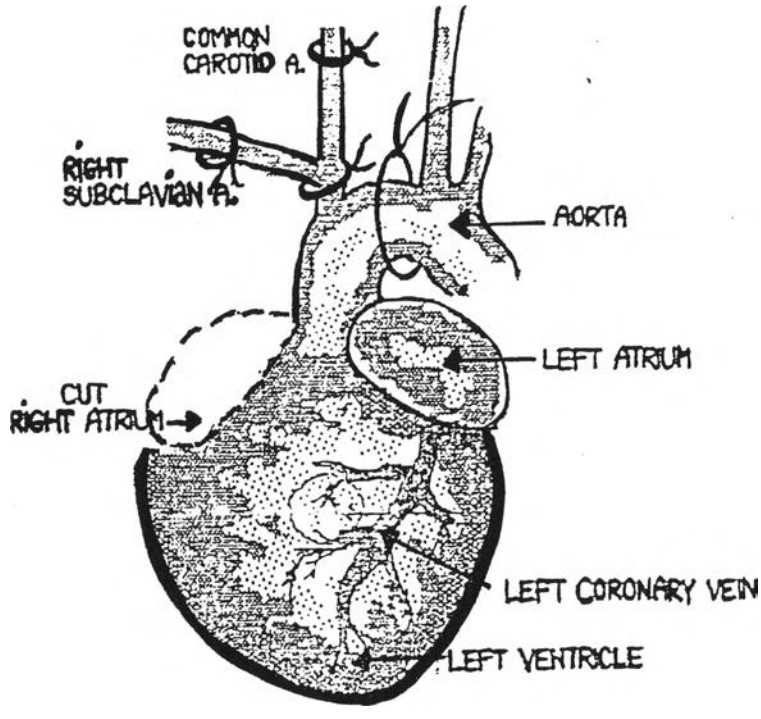


Figure 3.1 Cannulation procedure for perfusing the rat heart prior to isolation :

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

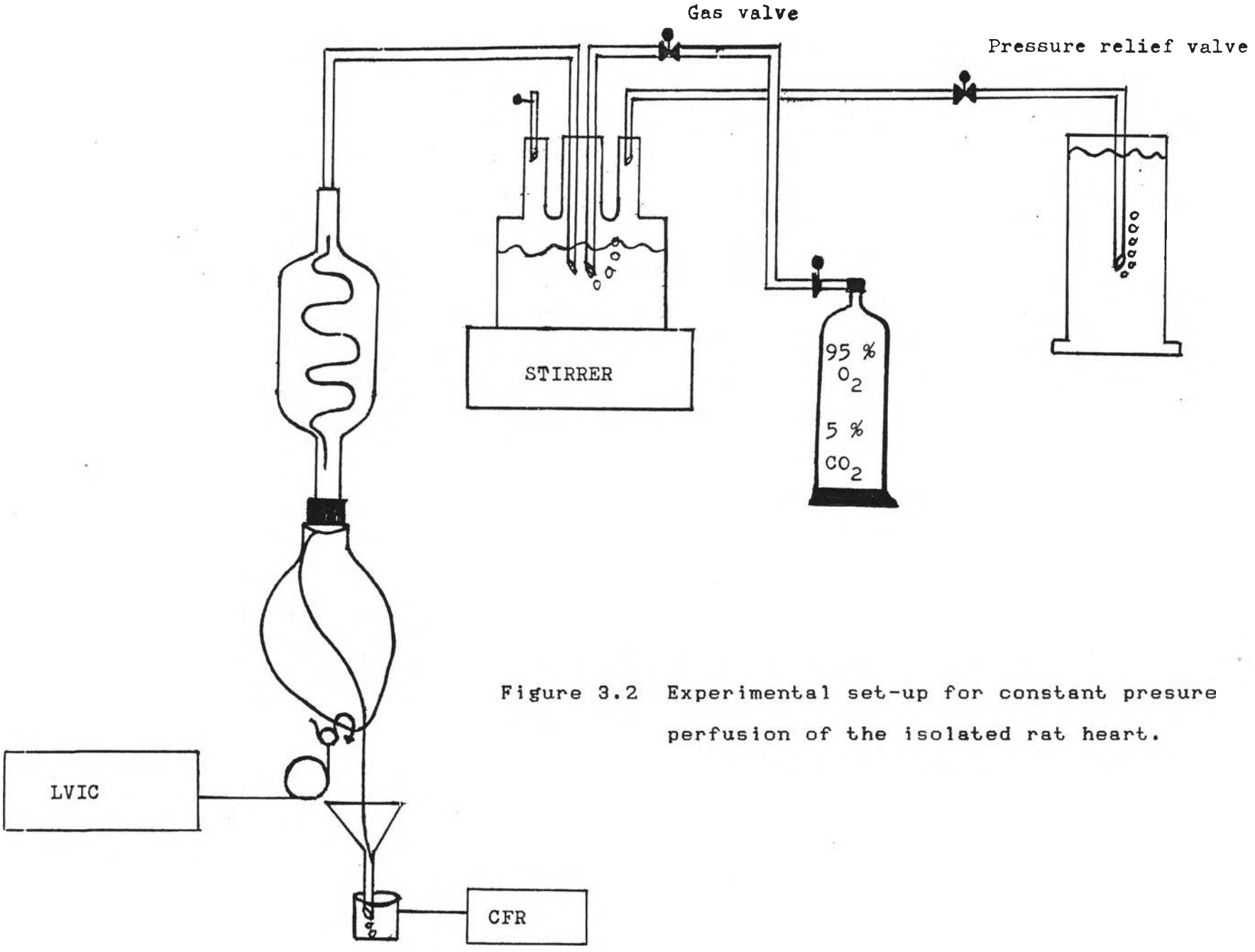


Figure 3.2 Experimental set-up for constant presure perfusion of the isolated rat heart.

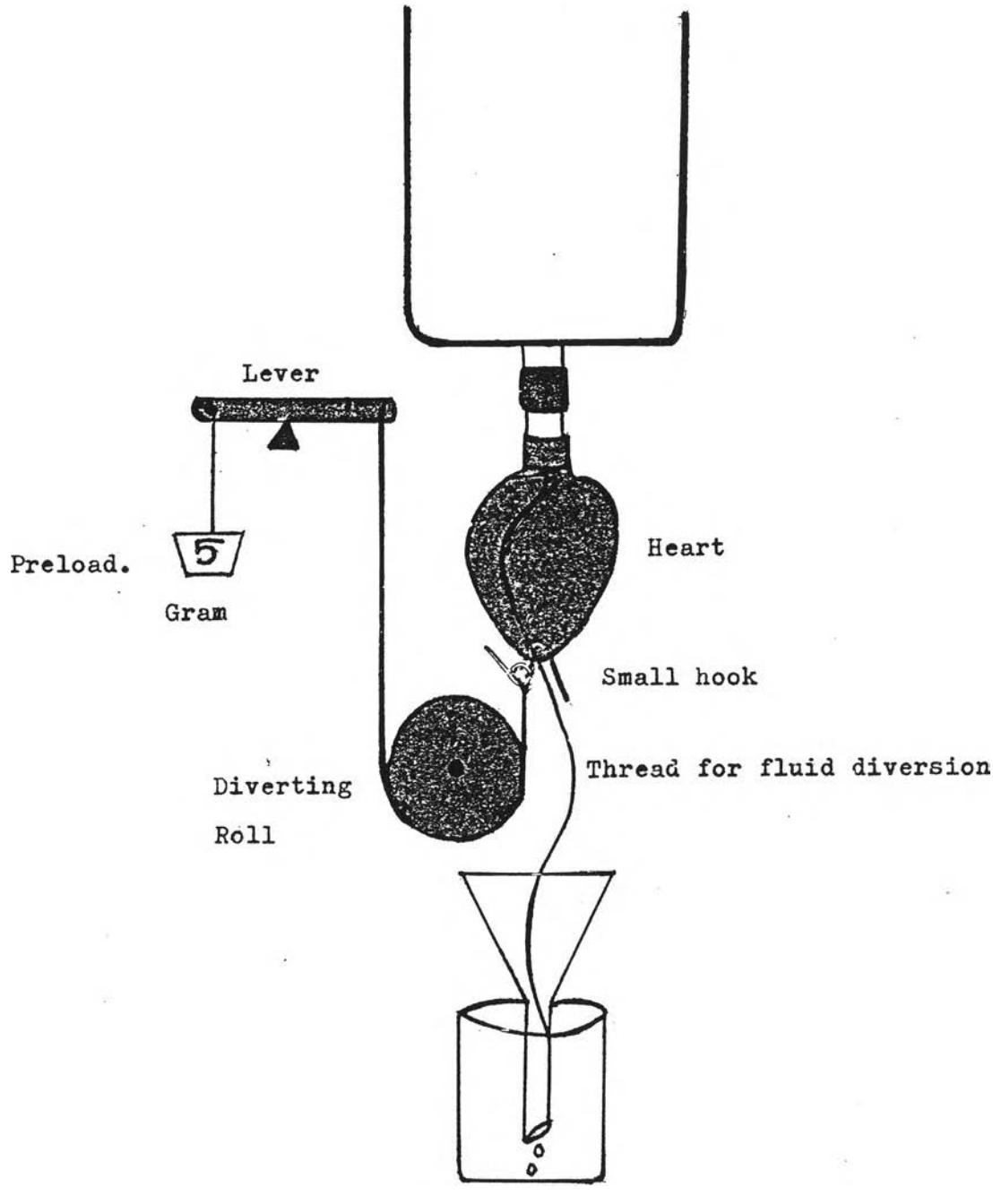


Figure 3.3 The force of contraction of each heart was measured with the preload of 5 gram.

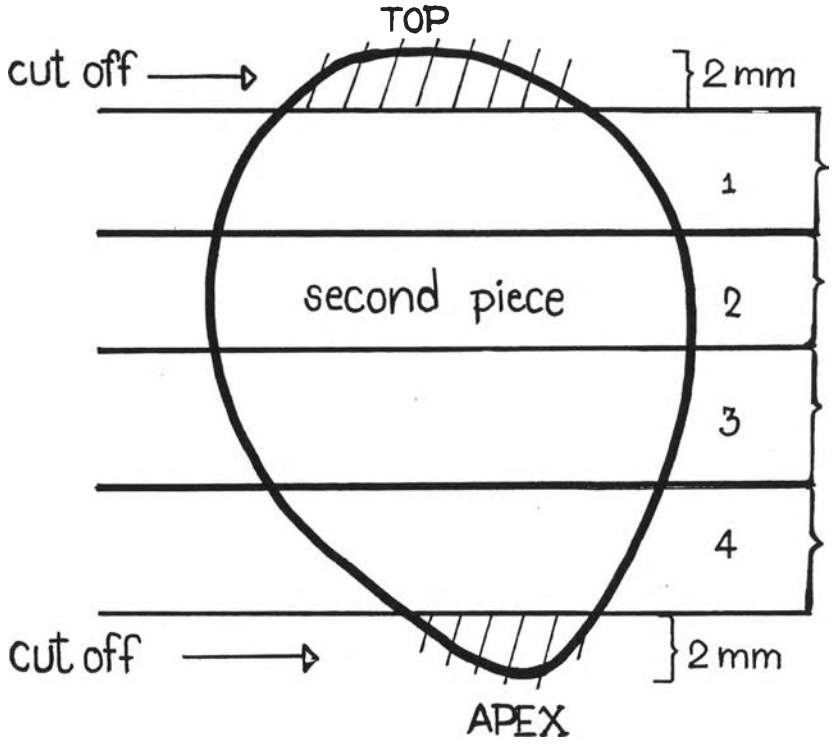


Figure 3.4 The heart was divided equally into four pieces. The thickness of each piece was about 3-4 mm. The second piece was then fixed by Eosin and Hematotoxylin method for further morphological examinations.

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 junctions. The walls of left ventricle and IVS were separated by this xy-line.