CHAPTER V

HEART AND RENIN.

1. MATERIALS.

Pure crystalline hormone six-times recrystallized (Imoto Pure Drug Co., Tokyo) containing 20.2 units/kg was used in preparing a stock solution 220 mg/ml in slight acidification using 1/20 hydrochloric acid (1 - 2 drops) and stored under refrigeration.

In this study, 2 mg/kg-dose was used. Drobner & Fisher (1954) had reported this dose to demonstrate maximal effect on rate of glucose utilization in the isolated perfused rat heart, subsequently confirmed by other reports (Fisher & Lindsey, 1955; Fisher & Williams, 1956b; Bonn et al., 1961).

2. SUBSTRATES (metabolites).

Pure crystalline substrate, obtained as g-oxaloacetate (E. Merck, Darmstadt, W. Germany) was used to prepare a stock solution 220 mg/ml. The dose added to the perfusate was 1 mg/ml and the calcium concentration in the buffer, 0.415 mg/l (half of the original Krebs-Ringer buffer) was buffered to 1.270 mg/l.

Drobner & Williams (1954) had reported the use of 1 mg/ml substrate to elucidate metabolic effects on the myocardial metabolism of carbohydrates in the isolated perfused rat heart, and had demonstrated the dependence of glycogenesis on calcium concentration at the site of the contractile element, to produce their maximal effects, and had suggested this change in calcium concentration.
3. METHADONE

Methadone hydrochloride, obtained as pure crystalline salt (Saffrania la Roche, Basle, Switzerland) was used in preparing a stock solution of strength 100 µg/ml and preserved under refrigeration. The guide-lines for dose-administration in a perfusion system of this order, were not available. Calculations were partly based on therapeutic weight limits for adult human administrations and partly on the reports of Hidrich & Felton (1949) on human toxicity studies, since the survival of this toxicology was to attempt an explanation on the mechanism of myocardial toxicity of methadone. Methadone hydrochloride was used in two doses in separate series of experiments: 1 µg/ml and 2 µg/ml; the latter dose is in conformity with adequate myocardial concentrations of methadone as reported by Farmer & Detwill (1949) in their studies on the distribution of methadone in tissues.

b. METHADONE HYDROCHLORIDE

Dextro-methadone dihydrochloride, obtained as pure crystalline salt (Saffrania la Roche, Basle, Switzerland) was used in preparing a stock solution of strength 100 µg/ml and preserved under refrigeration. Since the cardiovascular effects and toxicities are constantly under comparison with that of methadone, the dose standard was that of methadone, the dose standard was that of methadone, viz., 2 µg/ml., to facilitate comparison of their metabolic effects on the myocardium.
5. EXPERIMENTAL

Pure Pregnyl hydrochloride powder (methoxime) obtained from Imperial Chemical Industries, U.K., was used to prepare a stock solution - 2 mg/ml with slight warming which aided the sublimation of the compound (Johnston, 1963) and the solution was thereafter preserved under refrigeration. A concentration of 10 mg/kg-powder was used in this study, calculated as a comparable dose weight-for-weight to the dose of 1 mg/kg employed by Glotman & Masters (1967) and Masters & Glotman (1967) in their studies on cardiac metabolism of carbohydrates in dogs; this dose was also compatible with the clinically employed dose for its anti-anaphylactic activity.

6. RESULTS

Pure Esperin powder (mesylate) as Esperin sodium obtained from Upjohn International Inc., USA, conforming to the U.S.P. requirements of at least 180 units of anticoagulant activity per mg, was used to prepare a stock solution of 40 units/ml and preserved under refrigeration. The concentrations used in these studies were a total dose of 6 units and 8 units in the total perfusion volume in two separate series of hearts. This was calculated as the standard dose effectively used by Treidler & Johnson (1964) clinically, in the treatment of acute thrombotic conditions. Their total dose of 60,000 units per day in the average 70 kg, weighing adult was projected into
the requirements for the average weight of rat heart in this study, whereby doses of 1 unit (therapeutic dose) and 3 units were studied.

7. **EPINEPHRINE (Ephedrine, Eprocaine) **

Epinephrine obtained as a pure hydrochloride monohydrate salt from Actera Pharmaceutical Products Inc., U.S.A., was used to prepare a stock solution of strength 1 mg/ml and preserved under refrigeration. The concentration used in these studies was 20 mg/ml which was comparable to the dose employed by Mitchell & Bevan (1956) in dogs as an anti-arrhythmic agent in arrhythmias of mechanical stimulation, and was also in keeping with the dose used by Gordon & Steinhouse (1955) in dogs with ventricular stimulation induced by coronary artery ligature and hypoxemia.