CHAPTER - III

METHODS

The isolated perfused rat heart is a particularly attractive preparation for studying drug effects on cardiac muscle metabolism, for the following reasons:

1. It can be maintained visibly functioning, the performance during perfusion being monitored adequately by counting the heart rate and visually estimating the amplitude of contraction. (Cade et al., 1948).

2. The perfusion fluid reaches the myocardial cells along the normal channels.

3. The nature of the perfusion fluid can be varied at will, with the addition of the required concentrations of the drug under study.

4. The assembly, in its simplicity, compatible with efficiency, as employed in this study, has the advantage of being used, cleaned and re-set for carrying out as many as 3 to 5 heart perfusions per day under controlled conditions.

A. PREPARATION OF ANIMALS:

Male albino rats of an inbred strain of this department were used. The rat colony was maintained on a diet formulated by
TABLE - 23

RESEARCH RABBIT

(Department of Pharmacology)

INGREDIENTS PER KG OF STOCK FOOD PREPARED

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>White meat (potted)</td>
<td>780.00 g</td>
</tr>
<tr>
<td>Groundnut oil (potted)</td>
<td>156.00 g</td>
</tr>
<tr>
<td>(de-oiled)</td>
<td></td>
</tr>
<tr>
<td>Key fish (potted)</td>
<td>78.00 g</td>
</tr>
<tr>
<td>Groundnut oil</td>
<td>90.00 m</td>
</tr>
<tr>
<td>Sheep liver oil</td>
<td>5.00 m</td>
</tr>
</tbody>
</table>

Table

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monosodium hydrogen phosphate</td>
<td>1.00 g</td>
</tr>
<tr>
<td>Disodium hydrogen phosphate</td>
<td>1.50 g</td>
</tr>
<tr>
<td>Citric acid lactate</td>
<td>2.00 g</td>
</tr>
<tr>
<td>Iron ammonium citrate</td>
<td>0.15 g</td>
</tr>
</tbody>
</table>

Minerals (Chem Laboratories, India)

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>1.50 mg</td>
</tr>
<tr>
<td>Copper</td>
<td>1.50 mg</td>
</tr>
<tr>
<td>Iodine</td>
<td>25.00 mg</td>
</tr>
<tr>
<td>Chromium</td>
<td>0.50 mg</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.10 mg</td>
</tr>
<tr>
<td>Vitamin B1</td>
<td>9.00 mg</td>
</tr>
</tbody>
</table>

Prepared as balanced for content of carbohydrate, protein, fat, vitamins and minerals.

*Groundnut - synonymous with Pearson
this department as shown in Fig. 11. The results of this diet have already been reported from this laboratory (Christopher & Mason, 1966). Such a formulation became a necessity (instead of the standard commercial diets, the availability and reliability of which were dubious) and this was fulfilled from easily and locally available ingredients. The results have been uniformly satisfactory, with a steady gain in weight, the usual course being a rise of 25 grams per week during the first 6 weeks and 15 grams per week thereafter till the required weight-range for this study was attained.

B. PREPARATION ASSEMBLY

The perfusion assembly was designed to maintain a continuous recirculating perfusion system. The perfusion assembly is as shown in Fig. 11. The assembly was designed and fabricated in this department using a conventional 40-GUJJ isolated organ bath, mounted on a stand, and all the glass parts were designed and blown to specifications.

The buffer prepared for the day was initially stabilized with oxygen-saturated saline mixture for 15 minutes and then introduced into the perfusion system; thereafter it was evaporated. The volume employed was 25.9 ± 0.3 ml. Hearts inserted in the chamber received the buffer through the cannulated aorta, passed through the accessory arteries and veins and returned to the aorta and expelled through the great veins. The emerging
CONTINUOUS PERFUSION RECURRENTATION ASSEMBLY

FIGURE - 12
pericardial was collected by theCsummer-glass funnelled base
3 x 7 cm. internal dimensions. The funnelled chamber (heart
chamber) was closed by a rubber ring through which the sample
was held, which closed-off the re-circulating unit from the
atmosphere. The rubber ring also provided apertures for
(a) oxygenation and (b) a side-arm to act as gas-exit through
which a polyethylene tubing 1 cm gauge could be passed to
draw periodic samples of the buffer.

The effluent督查es from the heart chamber were carried through
a rubber-tying®, threaded through the rotor-motor of the
portable blood pump (AMINO-model No. 5-525, American Instrument
Co., Silver Spring, Maryland, USA) and carried to a constant
temperature bath above a bubble trap was located to prevent
bubbles from entering the sample; the bubble-trap was
connected to a side-arm of a mercury manometer. Preliminary
studies showed that when the temperature in the constant
temperature bath was maintained at 37°C, the temperature of
the buffer arriving at the sample was 31°C. The bubble-trap

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* TOSCA brand blood latex non-sensitive rubber-tubing gauge
1/8" x 1/8". American Hospital Supply Corporation, Evanston,
 Illinois, USA. A biologically inert tubing is very essen-
tial, since some types of rubber tubing incorporate organic
the during their manufacture, and this could depress cardiac
function within 30 minutes (Baylor et al., 1962). The rubber
tubing was replaced after three

pericardial.
time served as a petty reservoir for the circuit. The perfusion pressure of $25 \pm 3$ mm.Hg was used to produce adequate coronary flow, in conformity with earlier works.

After each perfusion, the interior of the re-circulating unit was thoroughly washed with distilled water, using the pump itself under high pressure to flush out the circuit, and finally flushed out with the buffer. A small volume of fluid was usually left after closing the unit (approx. 0.5 ml) which was a constant cover of less than 1% of the total volume reintroduced for each perfusion, and therefore no correction was deemed necessary (Nerem et al., 1967).

6. EXPERIMENTAL

The bicarbonate buffer of Erbe & Ruzicka (1956) (Figs. 19) was employed with two modifications. The calcium and magnesium concentrations were halved, recommended by earlier workers (Webb et al., 1961; Boyle, 1961; Zacharias, 1961) as necessary to maintain efficiency of contractile tissues over prolonged periods. The suggestion to reduce calcium concentration was made even earlier (Boyle & Casey, 1961).

The buffer for each day was made up from stock solutions of the various components, preserved under refrigeration, according to the procedure advocated by Tschetsch, Krelle & Schaffner (1957) and the substrate was finally added before equilibrating with
<table>
<thead>
<tr>
<th>Substance</th>
<th>Molecular Weight</th>
<th>g/litre</th>
<th>mEq/litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>58.448</td>
<td>6.9252</td>
<td>220.52</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>84.009</td>
<td>2.0929</td>
<td>64.92</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>74.997</td>
<td>0.3523</td>
<td>1.74</td>
</tr>
<tr>
<td>Potassium dihydrogen</td>
<td>136.092</td>
<td>0.1614</td>
<td>1.19</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>206.372</td>
<td>0.1967</td>
<td>1.27</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>246.386</td>
<td>0.1142</td>
<td>0.89</td>
</tr>
</tbody>
</table>
A gas mixture of 9% oxygen + 91% carbon dioxide, and the pH determined on a Cambridge pH meter to read 7.4. After additions of drugs, the pH was verified and the buffering capacity was found adequate to maintain the pH at about 7.4.

The substrate offered was glucose, since the study was focused on the effects of drugs on the various parameters of glucose metabolism measurable in this laboratory. Glucose (purer grade) from the Burman, India) Lot. no. 290.12, was used in concentrations of 150 mg/100 ml, as was done by earlier workers (Hochan & Hilder, 1939; Noyes et al., 1961; Vealway et al., 1966). In a few selected experiments, succinate (Furia, Sigma Chemical Co., St. Louis, Missouri, USA) of Lot. no. 246.1, was offered as an alternate medium in concentrations of 0.1 ml (Hillman & Bots, 1968).

2. MATERIALS

Rats weighing 250-375 g, fasted over 24 hours-water provided ad libitum were sacrificed by a blow on the head. No anticoagulant was used. The thoracic cavity was opened by a mid-line incision, stretched wide by retractor, after which the heart was gently lifted between fore-finger and thumb, the great vessels severed, making sure that as much of the aorta and the branch arteries of the arch were retained as possible in the removed heart. The heart was then placed in ice-cold buffer at 4° C when all contractions ceased in less than a minute.
The heart was then mounted on a ground annula, filled with buffer and perfused from an elevated variable bottle acting as reservoir for the buffer. The whole process from dissection to initial washing, took less than 2 minutes. The heart was washed out with the buffer under hydraulic pressure equivalent to 15 mm Hg and at a temperature of 4° C. The heart which had ceased beating while in the cold buffer, now began to contract, and coronary flow resumed usually in less than twenty seconds. This initial wash-out is referred to as the 'preliminary perfusion' or 'pre-perfusion', and this usually lasted about 3 to 10 minutes. Pre-perfusion was useful for the following purposes:

(a) washing out all blood or possible clots in the coronary vessels;

(b) provides facility for dissecting out other unnecessary tissue-like fragments of pericardium and appendages of small vessels attached to the arch of the aorta, and helped in assessing the suitability of the length of the aorta cannulated;

(c) stabilized the preparation to undertake a steady performance over the total perfusion period.
(d) The heart functioning normally for 20 minutes using the pre-purification medium which is substrate free, reaches a stable plateau from which all mechanical changes can be measured in evaluating the changes obtained by perfusing thereafter with known quantities of the substrate.

After the pre-purification in the wash-out system, the heart was rapidly transferred to the closed re-circulating assembly. The assembly was set in operation at least 20 minutes before the heart was transferred, by being filled with the desired volume of buffer, and equilibrated. The heart was maintained under continuous perfusion.

II. PREPARATION

The total duration of perfusion of each heart in the closed re-circulating system was 75 minutes. There has been considerable variation in the durations of perfusion by various workers. Studies on carbohydrate metabolism in all the parameters have been studied by Ogil (1953) using 20 minutes, Fisher & Lindsey (1953) and Machen & Fisher (1953) 75 minutes and Young et al. (1963) 60 minutes of perfusion. Hearts were studied in this series for 75 minutes, to permit drug action on the myocardial cell and the metabolism were adequately, and the time course of events were as indicated in Fig. 15.
FIGURE: 14

TIME COURSE OF VARIOUS EVENTS FOR RAT HEART PERFUSION

All figures indicate = minutes

A = Remove and mount heart
B = Perfuse without substrate using buffer (Pre-perfusion)
C = "Stabilisation" period (when indicated)
D = Perfuse with substrate (and drug) Perfusion Time
E = Sampling schedule (when performed)