A series of over 300 hearts have been employed in this study. A large number of perfusions have been done initially in this closed recirculating system for the standard 75-minute period to standardize the biological and mechanical factors involved in dissection, mounting of hearts, validity of the perfusion assembly and in checking on the perfusion prerequisites such as cardiac contractility, cardiac rate, coronary flow rate and perfusion pressure using glucose (0.25 wt) 150 mg/100 ml as the standard substrate.

Thereafter studies on carbohydrate metabolism were undertaken.

a. Indices of Carbohydrate Metabolism

The following indices of carbohydrate metabolism were measured:

1. Glucose uptake from the perfusate
2. Oxygen content of heart muscle
3. Lactate content of heart muscle
4. Lactate content of perfusate at the end of perfusion. (Lactate output)
5. Pyruvate content of heart muscle
6. Pyruvate content of perfusate at the end of perfusion (Pyruvate output)
b. **Materials for Studies Concerned for the Study of Substrate Utilization**

Studies on the rate of uptake of glucose over a series of isolated perfused hearts establish the functional integrity of the tissue. The permeability of this sugar into the tissue is altered considerably by insulin and other agents and the study of **glucose-metabolism** in the presence of various drugs, as designed in this study would therefore reveal the effects of these drugs on the sugar permeability in the perfused heart.

A portion of the glucose taken up by the tissue is converted into glycogen, by the process of glycogenesis, and a portion broken down and utilized for energy. **Measurement of glycogen** in the hearts perfused with glucose substrate for 75 minutes served as *paradigm* to evaluate normal pattern of changes in this parameter contributed by the glucose taken up by an actively functioning tissue. Subsequent perfusions of a similar duration and substrate-content, in the presence of drugs, would reveal their effects on glycogenolysis or glycogenolysis (as the case may be) when compared with the controls.

Measurements of lactate and pyruvate were made to study the influence of drugs on glycogen, this term indicating the breakdown of glucose to pyruvic acid and lactic acid. These observations will assist in segregating the metabolism of carbohydrates into the two phases: anaerobic and aerobic,
though this distinction is actually somewhat arbitrary. The reactions of glycolysis are the same in the presence of oxygen, as in its absence, except in extent and end-products. During the anaerobic cycle of events, Mitochondrial Adenosine Diphosphate (ADP) - a respiratory chain component for those respiration - which is normally rejuvenated to activity by resuscitation, is impaired; NAD is re-oxidized by the reaction involving the reduction of pyruvate to lactate. Lactate rather than pyruvate thus becomes the principal end-product of glycolysis under anaerobic cycle of events.

Measurement of lactate therefore, in both heart muscle and in the periphery (after 75 minutes of perfusion) in central experiments and in the presence of drugs, will indicate the effect of the drug on glycolysis. The oxidation of glucose to lactate, as the end-product of glycolysis (as would be apt to occur under anaerobiosis) limits the amount of energy available to the cell; consequently, more glucose may need to be taken up or more glycogen may need to be broken down.

From the standpoint of energy metabolism, the most important of which is the Glycolytic Pathway to pyruvic acid and lactate acid, (the end-products of glucose metabolism) it is mainly pyruvic acid (obtained also from the reaction:

\[
\text{Lactate acid} \xrightarrow{\text{Pyruvate dehydrogenase}} \text{Pyruvic acid}
\] which is oxidatively decarboxylated through an aldol to acetic acid. This ten-carbon compound.
("active acetate") enters the aerobic citrate acid cycle of heart readily, and is completely degraded to \( \text{Ac} \) and \( \text{Py} \), thus completely metabolizing energy for the principal purpose of producing energy for the myocardial cellular metabolism.

Therefore, estimations of pyruvate content in both heart muscle and the perfusate after 75 minutes of perfusion provides an insight to some degree of the aerobic pathway undertaken by the heart muscle, and study of the influence of drugs on this parameter, compared to control experiments will help in assessing their effects on the aerobic cycle of glucose metabolism. The plan thus envisaged is summarised in Fig. 20.

6. Preliminary Studies

Central values were recorded for glucose-contents with periodic sampling at 10, 45, 60 and 75 minutes, drawing 0.2 ml each time, with appropriate corrections for volume changes due to sampling. Central values were obtained for glycogen, lactate and pyruvate content in different groups of unperfused rat hearts immediately after decapitation, and in perfused hearts after 75 minutes perfusion with glucose-saturated buffer.

Glycogen and lactate content of hearts were obtained also after 30 minutes perfusion with substrate-free buffer, to facilitate the evaluation of net changes in glycogen and lactate and to stabilise the preparation for continuous perfusion thereafter (Cople et al., 1963; Tendler et al., 1963).
PARAMETERS MEASURED IN PERFUSATE AND HEART MUSCLE AS INDICES OF CARBOHYDRATE METABOLISM IN THE ISOLATED PERFUSED RAT HEART
This was not deemed necessary for pyruvate because of its new
stability and the ready change to lactate and during activity
in the beating heart (Gamblin et al., 1983).

Drug studies were then begun. All the parameters listed, were
measured, with each of the drugs: Insulin, Lactate, Lactase,
Strychnine, Loperamid, and Hyoscine. While
phosphocreatine measurements and gynogen estimations were
done on the same heart, lactate and pyruvate estimations were
done in different groups of hearts.

Insulin was employed in this study with a two-fold purpose:

(a) Since the effects of Insulin on a preparation of this
nature has been extensively reported, using this well-
established activator of carbohydrate metabolism helped
in characterising the experimental procedure and the
estimation methods.

(b) Insulin was used in combinations with drugs: Lactase,
Strychnine and Propocain, to be able to study any
possible relationship between their metabolite effects
which could consequently help in explaining the myocardial
effects of the drug alone. It was also deemed possible,
that, Insulin, whose effects are well known, may help to
unmask the effects of these agents whose metabolite effects
are relatively obscure.
Cocaine, used in this study, was reported for its metabolic effects on the heart, earlier by Brandberg & Williams (1964) and it was helpful to use their results as a comparison with those obtained in this thesis, in characterizing the validity of the preparation and procedure. It was also considered useful as a basis of comparison for the effects of other drugs; the therapeutically beneficial effects of cocaine and its metabolic effects can readily be correlated. Therefore, the metabolic effects produced by the drugs under investigation could have a comparable parameter to express their beneficial or toxic effects as the case may be.