Chapter-1

Introduction and Rationale of Work
INTRODUCTION

The present work was carried to study certain biochemical, histopathological and thermodynamical properties of pericardium. The biochemical parameters included estimation of total lipids, cholesterol, phospholipid, triglyceride free fatty acid and trace metal elements viz.; Nickel, Cobalt, Copper, Chromium, Iron, Zinc, Manganese, Calcium and Magnesium. Estimation of calcium also included calcium uptake by porcine and bovine (buffalo) pericardium. Histological studies were mainly directed to scanning electron microscopic examination of bovine pericardium. However, routine histological examination of pericardium stained with Hematoxylin and Eosin (HE) was also performed.

Main emphasis was given to the thermodynamic study of pericardium based on the laws of irreversible thermodynamic developed by Kobatake et al. (86,97) and Nagasawa et al. (98).

The experimental set up has been shown in figure 1 and explained in detail under heading "Materials and Methods". Briefly, it may be mentioned that a piece of pericardial membrane measuring approximately 2 cm in diameter was removed immediately after the animal was slain and put in Ringer's solution to ensure its viability. It was later
interposed between two glass chambers containing the same electrolytic solutions with different concentrations. The electrical potential generated across the membrane was recorded and transport number, effective fixed charge density and permselectivity were calculated on the basis of the laws of irreversible thermodynamics.

The rationale for this plan of work is as follows:

1. Kobatake et al. and Nagasawa et al. developed certain theories of membrane potential based on non-equilibrium thermodynamics for synthetic membranes, such as ion-exchanger membranes, bilayer lipid membranes etc. prepared in laboratories from various chemical substances. The transport of ions across these artificial membranes were found to be governed by these laws of irreversible thermodynamics which has been confirmed by the investigators world over. Since the ultimate aim of the fundamental research is its application in the services of the mankind, it is natural to examine the applicability of these laws of irreversible thermodynamics to biological system. Unfortunately, however this aspect did not receive the due attention of scientist that it deserved. The reason for this was mainly the lack of interaction between biologists and
physical chemists. Experimental model for study of irreversible thermodynamics designed by various investigators consisted of separation of two solutions by a sheet of artificial membrane. Some physical chemists isolated the egg shell membrane by dissolving the egg shell with weak acid in an attempt to obtain a biological membrane of sufficient area to be able to conduct thermodynamical studies. But the chemical nature of the membrane got altered on treatment with weak acid and the results could not be applied to living tissues. Ussing (144-145, 171) tried to examine the applicability of the laws of irreversible thermodynamics with the help of frog skin. But the frog skin was too thick to be a suitable structure for such studies.

Biological chemist on the other hand concentrated mainly on studies on biochemical, metabolic and histological studies on isolated cell membranes. But the irreversible thermodynamical studies were not possible on isolated cell membranes because, for such studies a thin sheet of membrane was required to suit the experimental set up developed by Kobatake et al.

We, therefore, opted to compromise between these two extremes - viz; relatively thick frog skin and
inappropriate size of isolated cell membrane by conducting the thermodynamical studies on pericardial membrane which is quite a thin structure as compared to frog skin and also available in the form of a sheet to suit the experimental set up developed by Kobatake et al.

2. The importance of characterizing the composition of biological membrane and its behaviour are obvious. Such studies will be of great clinical significance. The biological membranes not only form covering of various cells, tissues and organs but also govern the movement of nutrients, toxins and other substances across the membrane. Any change in the structure and function of a membrane will adversely affect the integrity of the cell and ultimately, that of the living organism itself. Unfortunately, the laws of irreversible thermodynamics which are expected to influence this critical function of cell membrane could not be undertaken because of want of inappropriate experimental model as has been explained above.

Obviously, studies on pericardial membrane can not be transpolated in toto to the function of individual cell membrane and this is the limitation of such studies. But such studies on pericardial membrane will definitely form a
better model as compared to frog skin for studies on biological system, and such studies will definitely throw light on the behaviour of biological membrane as compared to artificial membranes.

3. Pericardium is a sac-like structure consisting of fibrous tissues and cells and surrounds the heart. Apart from affording support to the heart it also influences movement of fluid and other substances in the pericardial sac. Pericardium has been used to make bioprosthetic heart valves. In many cases, specially in children, it has been observed to get calcified, thus, limiting the scope of pericardial valves. Because of these significance, pericardium was selected as a very suitable membrane for examination of thermodynamical properties.

4. The functional behaviour of biological membrane will be influenced by its chemical composition and structure. Unfortunately the biochemical composition of pericardium has not received due attention with this aspect in mind. Routine light microscopic structure of pericardium is well known but the electron microscopic structure of pericardium has not been studied in detail. Such studies are specially lacking on bovine pericardium. Since bovine pericardium will be a
very suitable structure for conducting the thermodynamical studies, it was considered appropriate to examine light microscopic and electron microscopic structure of bovine pericardium and to characterize its biochemical nature. We have confined our studies to examination of the surface of the bovine pericardium (scanning electron microscopic studies) which will be important for its transport function. For biochemical studies we have selected the parameters which are most likely to influence the transport function as well.

5. During the course of our studies we observed that pericardium imbibes calcium from the immersing fluid. We also noted from the literature that pericardial bioprosthetic valves (which are usually obtained from porcine pericardium) get calcified with the passage of time. We wanted to know whether this calcification is species related or not. The plan of these experiments has been described in detail under 'Materials and Methods' and the findings are discussed under 'Results and Discussion'.

6. We have also described the difficulties and limitations of our experimental model, but the results are significant and we hope that the findings of this work will form the
basis for future research on this important and interesting field of irreversible thermodynamics with respect to biological membranes.

A detail review on these aspects has been presented. The results have been critically analysed under 'Results and Discussion'.