Chapter I: INTRODUCTION

The study of abnormal haemoglobins ever since the discovery of Hb S in 1949 (Pauling et al, 1949) has evinced paramount interest amongst haematologists, biochemists, geneticists as well as clinicians. Haemoglobinopathy, though a new entrant in the field of clinical medicine has already created sufficient amount of stir among clinicians, and reports are being recorded frequently from different parts of the globe of the existence of one or other abnormal haemoglobin and their interactions. Problems have arisen as to the nomenclature of the newfinds. In course of the VIIIth International Congress of Haematology, September 1960, in Tokyo, experts met to discuss the nomenclature of the human haemoglobin. The field is expanding so rapidly that it is not possible even at an International Congress to obtain a full representation of all disciplines and groups of workers. So the Congress laid down certain recommendations (Report: Nomenclature of abnormal haemoglobins, 1961) regarding the nomenclature and invited criticisms. Hence it is obvious that the nomenclature of human haemoglobins which has posed a problem is yet to be solved.

Congenital haemolytic anaemias have been a great challenge to the clinicians through years. Homozygous thalassaemia, a congenital haemolytic disorder, characterised by the persistence of high foetal haemoglobin usually proves fatal before puberty. With the discovery of abnormal haemoglobins, many cases of homozygous thalassaemia, after renewed characterisation studies
have turned out to be in interaction with one or other abnormal haemoglobin (usually Hbs E and S). There are now several impelling reasons for looking into these diseases in a more comprehensive manner with the help of physiologists, biochemists, pathologists and immunologists.

Current literatures show that the investigations have been directed all over the world to study the haemoglobin and red cell membrane from various aspects. Reports seem scanty and frequently contradictory as far as the oxygen haemoglobin reaction in haemoglobinopathies is concerned. The relation of oxygen affinity of Hb F to that of Hb A is by no means certain (Allen et al 1955). Kirschbaum (1964) noted that oxygen capacity of the foetal blood was same as that of the blood of human adult, whereas Abrahamov and Smith (1959) observed the reduction in the oxygen capacity in direct coombs-positive foetal erythrocytes. Likewise, Riggs and Wells (1961) reported that Hb S had a lower affinity than Hb A in agreement with Becklake et al (1955) but in contrast to Wyman and Allen (1951) who found no difference.

In a recent work Huisman and Schillhorn (1964) have shown that oxygen affinity of red blood cell haemolysate obtained from adults known to be traits of various haemoglobinopathies was identical with that of a haemolysate of normal adult red blood cell except in HbPunjab trait where the oxygen affinity was increased. This observation is not in full agreement with an earlier investigation (Sarkar and Nagchandhuri, 1961).

This work has been taken up with a view to investigate further the oxygenation of haemoglobin in haemoglobinopathies. Oxygen capacity has been determined in whole blood samples as well as in their haemoglobin solutions.
in conditions of haemoglobinopathies as well as in normal subjects and patients suffering from other anaemias. The purpose of studying oxygen capacity in haemoglobin solutions is to investigate whether samples containing abnormal haemoglobins have any role in altering the values of the oxygen capacity.

Intense study has been conducted in various laboratories to investigate the composition of the red cell membrane. There is evidence that the thickness of the red cell has got influence on the oxygen dissociation curve in anaemias (Valtis and Balkia, 1955; Horejsi and Komarkova, 1958, 1959). These informations prompted us to carry on further investigations on the red cell membrane or stroma in cases of haemoglobinopathies.

The stroma consists mainly of protein (40 - 60 %) and of lipid (10 - 12%). Only a few works have been published regarding the lipid content of the stroma (Erickson et al, 1957; Schwartz Tiene et al, 1955; Sarkar, 1961; De Gier and Van Deenen, 1964 and Micopolous et al, 1966). These reports are not always in agreement with each other.

Mucopolysaccharides and protein content of the stroma were also investigated. Mucopolysaccharides occur in epithelial and connective tissue. The level of mucopolysaccharides have frequently been expressed in terms of hexose and hexosamine content.

Estimation of hexosamine was done in the stroma of samples from haemoglobinopathies as also the nitrogen content. The value of total nitrogen also included non protein nitrogen. But usually the content of non protein nitrogen is very small as compared to the total nitrogen content. So the value for total nitrogen of stroma may be accounted for its total protein content.
No data were found in the literature concerning the concentration of hexosamine and protein in the stroma of samples from haemoglobinopathies.

In view of the evidence that red cell membrane can play a part in initiating haemolysis, a comprehensive study of the stroma has been taken up in the present investigation with regard to its total lipid, hexosamine and nitrogen contents, to throw some light on the mechanism of haemolysis in haemoglobinopathies.

Based on the above objectives the overall arrangement of the present research investigation comprises detailed analysis of the oxygen capacity, in whole blood and in haemoglobin solutions of the samples studied. Red cell membranes or stroma of these samples of blood have been isolated and thorough study has been made with regard to its lipid, hexosamine and protein contents.

Although the major emphasis has been laid upon the experimental work but to establish the definite diagnosis of the patients suffering from haemoglobinopathies and other anaemias, a thorough clinical investigation including the radiological, pathological and biochemical studies as well as the electrophoretic analysis of the haemoglobins has been conducted and has been reported in the Chapter IV of the thesis.

Thalassaemia, either in homozygous form or in combination with Haemoglobin E appears to be the commonest form of haemoglobinopathy in Bengal (Chatterjee, 1959; Chaudhuri et al, 1962; Chatterjea, 1966). The heterozygous thalassaemia in Bengalees approximates 3.7% (Chatterjea, 1969). Instances of Hb E-Thalassaemia disease have been reported from two centres of Calcutta (Chatterjea et al, 1957; Sarkar et al, 1959). So most of the
studies in our series have been conducted in homozygous thalassemia and Hb E-thalassaemia disease although a few blood samples containing other abnormal haemoglobins were collected from elsewhere for investigation.

Blood from normal subjects and samples of cord blood from full term infants have also been investigated for comparative study.