Haemoglobinopathy, a new entrant in the field of clinical medicine, has been a subject of tremendous interest from the point of view of research as well as treatment. Abnormal haemoglobins are being studied from different aspects, and the clinical syndromes associated with these haemoglobins have presented complicated clinical problems.

The diagnosis of a haemoglobinopathy in the present investigation had been made by noting the clinical signs and symptoms, and by pathological and biochemical investigations of which paper electrophoresis of haemoglobin and alkali denaturation test were important. Radiological investigations also came to the aid of the diagnosis.

Family studies also helped in the diagnosis. As the inheritance of thalassaemia major and Hb E-thalassaemia disease follows the principle of the Mendelian recessive pattern, the finding of expression of the thalassaemic gene and Hb E gene (as the case may be) in one or other parents would explain the genetic constitution of the patients. Thus S.G. (Serial No. 31) a patient with Hb E-thalassaemia had Hb E-gene from one of his parents and thalassaemic gene from another to explain his genetic constitution. Electrophoretic study of blood samples of his family members showed that the mother continued Hb E-trait. The thalassaemic gene in this patient came from his father and that was expressed by the presence of high Hb A2 content (19%) in his father’s blood. In thalassaemia major, a homozygous condition, each parent should have a thalassaemic gene to make up the genetic constitution. In all cases of thalassaemia major studied, the parents showed
high percentages of Hb A₂ on paper electrophoresis, the maximum being 19%. Aksoy et al. (1957) however pointed out that in thalassaemia traits the Hb A₂ percentage almost never exceeds twenty whereas in Hb E-trait carriers (A + E) the component invariably exceeds twenty percent. But Chatterjea et al. (1959) reported a higher percentage of Hb A₂ in thalassaemia traits on paper electrophoresis.

Signs and symptoms of thalassaemia major and Hb E-thalassaemia are the same. It has been observed that if thalassaemia is associated with Hb E, the course of the disease may be less rapid, so that some may live to adult life (Whitby & Britton, 1957). In the present series A.H. (Serial No. 57) was 30 years of age and had splenectomy done at the age of 29. The age of another patient was 17. (S.P., Serial No. 56).

It is known that in anaemic patients there occurs a state of hypoxia, and the oxygen capacity is diminished along with their haemoglobin content. The present investigations on oxygen capacity showed that the average values of the capacity in whole blood in various types of anaemias and haemoglobinopathies studied were reduced in comparison with those in normal subjects.

The average result of the oxygen combining capacity in normal subjects of the present series was 1.358 ml./gm. of Hb which was almost similar to the theoretical standard of 1.54 ml./gm. of Hb. The reduction in the capacity was maximum in Hb E-thalassaemia disease the average value being 1.15 ml./gm. of Hb. The average values of the capacity in thalassaemia major, in the thalassaemia minor and in other anaemias were 1.31, 1.16 and 1.23 respectively, all values expressed as ml./gm. of haemoglobin.

Reports are scanty in the literature about the determination of oxygen
capacity in anaemias particularly in haemoglobinopathies. But studies have been made about the oxyhaemoglobin dissociation curve in these conditions which show that there occurs shifting of the dissociation curve to the right (Chapter II, Section B). It means a reduction of percentage saturation haemoglobin at the particular partial pressure of oxygen. Several factors have been held responsible for the shift of the oxyhaemoglobin dissociation curve. Henderson (1928) regarded this shift in anaemia as a possible compensatory mechanism of the anaemic state. The shift is attributed to the relative acidity of red cells in anaemias (Green, 1949) which has previously been directly measured by other workers. Kennedy and Valtis (1954) have shown that the curve even after correction to standard cell pH remained shifted to the right. The only exception to this rule has been the blood of patients with spherocytic anaemias; and it has been suggested that this difference is due to greater thickness of spherocytic red cells.

From the results of study of the oxygen dissociation curves in anaemias and haemoglobinopathies, it can also be surmised that there is every possibility of the reduction of oxygen capacity as well when the blood samples of anaemias and haemoglobinopathies are exposed to the atmosphere for full saturation of its haemoglobin with oxygen.

Rodman et al (1960) has described the desirability of a reduction in the affinity of haemoglobin for oxygen in the form of a rightward displacement of the dissociation curve as it facilitates the extraction of oxygen from the haemoglobin by the tissues. Thus, when the haemoglobin content of the blood is normal, the oxygen needs of the tissues are readily met, and the body can afford the luxury of an inefficient extraction of oxygen which permits 80% to be returned to the lungs unused. In the presence of severe anaemia, however,
it is imperative that the tissues extract a greater percentage of haemoglobin-bound oxygen, since the absolute quantity carried is markedly reduced. A reduction in tissue oxygen tension will result in more nearly complete extraction. A marked reduction in tissue oxygen tension, however, will impair intracellular enzymatic processes, and a moderate reduction will leave much of the oxygen unextracted (over 50%). Under these circumstances, a decrease in the affinity of haemoglobin for oxygen would be desirable.

In the present series the average reduction in the oxygen capacity has been maximum in patients with Hb E-thalassaemia disease, being 1.15 ml./gm. of Hb. It is suggested that the blood replaced wholly by abnormal haemoglobins (in this instance, Hb E and Hb F) might have a lesser affinity for oxygen than the blood containing a mixture of normal adult and abnormal haemoglobins (Hbs A+F, as in thalassaemia major).

Rodman et al (1959) studied oxygen dissociation curve in anaemias 'in vivo'. Out of 12 patients studied whose haemoglobin was entirely or partially abnormal, they have observed arterial oxygen unsaturation in five patients, who had no normal haemoglobin and the oxyhaemoglobin dissociation curve is displaced to the right. Fraimow et al (1958) have suggested that shifting of oxyhaemoglobin dissociation curve in sickle cell anaemia is due to the defect related to the Hb S-containing red cells per se.

Information about the comparative study of reduction of oxygen capacity in different abnormal haemoglobins is very poor in the literature. Reports in this respect are sometimes contradictory. For example, Abrahamov and Smith (1959) observed the reduction in the oxygen capacity in direct
Coombs-positive foetal erythrocytes, whereas Kirohbaum (1964) noted no difference. Similarly, Becklake et al (1955) and later Riggs and Wells (1961) reported that Hb S had a lower oxygen affinity than Hb A, whereas Wyman and Allen (1951) found no difference. We found the reduction in oxygen capacity in the investigation of cord blood, thalassaemia syndromes and in samples containing Hb E and Hb F (Sarkar and Nag Chaudhuri, 1961). These discrepancies may result from very different experimental conditions as well as methods of handling the haemoglobin prior to the determination.

From the present investigation it is observed that the average value of oxygen capacity in Hb E-thalassaemia disease was lower than that of thalassaemia major. In the five cases of Hb E-thalassaemia disease studied the oxygen combining capacity ranged from 1.0 to 1.24 ml./gm. of Hb, with the average value of 1.15 ml./gm. of Hb and standard deviation ± 0.08. There seems to be a possibility of some defect in blood samples consisting wholly of abnormal haemoglobins.

The results of the determination of the oxygen capacity of haemoglobin solutions in different groups of patients were interesting. The average values of the capacity in these groups of patients were increased than their corresponding values in the whole blood samples. The average values of the capacity in haemoglobin solutions in various groups of patients studied were: in thalassaemia major, 2.21; in thalassaemia minor, 1.88; in Hb E-thalassaemia, 1.44 and in other anaemias, 1.77; all values were expressed as ml./gm. of Hb. The average value for normal subjects was 1.57 ml./gm. of Hb. The increase in the oxygen capacity in haemoglobin solution was maximum in thalassaemia.
major and minimum in Hb E-thalassaemia disease. In one case of thalassaemia major (Serial No. 16) the increase of the capacity was more than double its value in whole blood. The average value of the capacity in haemoglobin solution in Hb E-thalassaemia disease was minimum in this series though the reduction of the capacity in this group was maximum in whole blood samples. This difference may be due to a condition where the normal adult haemoglobin has been almost wholly replaced by abnormal haemoglobins.

In a recent work Huisman and Schillhorn (1984) have shown that red 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 where the oxygen affinity was increased. In our investigation the same was found to be increased in red cell haemolysate obtained from thalassaemia traits.

Hartridge and Roughton (1927) first reported in vitro measurements of the rates of uptake of oxygen and carbon monoxides by suspensions of sheep red blood corpuscles. They also showed that the time to reach 40% saturation was about 20 times longer in the red cell suspension than in the homogenous haemoglobin solutions of the same gas combining capacity as that of the above red cell suspension. They suggested two factors as possible explanation for this difference: (a) Resistance offered by the red cell membranes to the passage of dissolved oxygen; and (b) the finite thickness of the interior of the red cell. The first oxygen molecules to pass through the cell membrane tend to combine with haemoglobin in the outermost layers of the red cell, thus opposing a barrier of partially combined haemoglobin to the next oxygen molecules to enter the red cells. The results of study of Hartridge and Roughton (1927) though instructive do not give any information as to whether
the oxygen capacity shall be increased in haemoglobin solution. Moreover, search of literature on this condition could not give any clue. In haemoglobin solution the normal environment of erythrocytes (i.e. red cell membrane, plasma, etc.) is removed and the haemoglobin molecules are uniformly distributed in a homogenous solution like this. It is suggested that in this condition the haemoglobin shows greater affinity for oxygen than in whole blood thus increasing the oxygen capacity.

Moreover, the amount of total lipids present in the haemoglobin solution might also play some important part in the increased oxygen capacity of the haemoglobin. In solutions of adult haemoglobin the total lipids varied from 0.08 to 0.12, in thalassaemia major from 0.24 to 0.35 and in Hb E-thalassaemia disease from 0.25 to 0.39. All values are expressed as mg. present in 1 ml. of haemoglobin solution containing 1 gm.% of haemoglobin. The haemoglobinopathies showed high content of total lipids in their haemoglobin solutions, and it is more evident when the sample contains only abnormal haemoglobins.

The investigations on cord blood showed reduction in the oxygen capacity. The average value was 1.05 ml./gm. of Hb. Swierczewski and Minkowski (1956) have shown a mean reduction of about 5% in the oxygen capacity in cord blood than in the blood samples from the adults. It has also been suggested (Kravitz et al, 1956; Betke, 1954) that the reduction in the oxygen capacity might be due to presence of some percentage of the haemoglobin in the form of methaemoglobin. The results of the present investigation corroborate with the findings of the workers mentioned above, though the reduction of the capacity in the present series was of a greater degree.
In haemoglobin solutions of cord blood also there was an increase in the oxygen combining capacity and the average value was 1.50 ml./gm. of Hb. Smith (1959) has pointed out that although a solution of human foetal haemoglobin has an oxygen dissociation curve lying to the right of that characteristic of solution of maternal haemoglobin, human foetal blood (or a suspension of its washed erythrocytes) has a curve to the left of that of maternal blood. The affinity of erythrocytes for oxygen is affected not only by that of their haemoglobin, but also by the permeability of the cell walls containing the haemoglobin, and presumably by their chemical contents other than haemoglobin. The oxygen affinity of blood is also affected by extracellular factors such as the acidity and the carbon dioxide content of the plasma. Therefore it is highly suggestive that the oxygen combining characteristics of human foetal blood are not essentially properties of foetal haemoglobin. Allen et al (1953) have shown that maternal haemoglobin in solution is more saturated with oxygen than the foetal haemoglobin in solution at all oxygen pressures.

Cord blood samples were investigated for two purposes. The first was to study the oxygen capacity of the blood of new born infants. The second point was that the cord blood contained high amount of foetal haemoglobin. The blood samples of thalassaemia syndrome also contained high percentage of foetal haemoglobin. Despite certain differences amongst workers, the consensus of opinion is that the foetal haemoglobin present in new born infants are similar to foetal haemoglobin present in the thalassaemia syndrome. The present attempt was to assess the oxygen capacity of the cord blood and of blood from a thalassaemia patient, and to know whether there was any abnormality in this particular issue. No relationship could be found out between these two samples of blood and in haemoglobin solutions.
of these samples. Thus, the average values in whole blood of oxygen capacity of cord blood and of thalassaemia major were 1.05 and 1.31 ml/gm. Hb, although the average values of their foetal haemoglobin were almost same (45.8% and 45.6% respectively). Of course this is not enough evidence to comment on the foetal haemoglobin of cord blood and that of thalassaemia syndrome as there is question of percentages of foetal haemoglobin in each case studied as also the degree of transformation to methaemoglobin as referred to above.

Recent studies on oxygen dissociation curve in anaemias show that the thickness of red cell has got influence on this curve (Váltis and Baikie, 1955). In further studies the factors contained in the membranes of the red blood cells have also been held responsible for the change of shape of the dissociation curve (Horejsi and Komarkova, 1958, 1959). It has been shown that the dissociation curve of crystalline haemoglobin is not influenced by glutathione contrast to that of native blood but the effect appears after addition of stroma of the red cells.

The study of the above literatures reveal that in cases of anaemias there are factors in the red cell membrane which may inhibit the passage of oxygen into the red cells for combination with haemoglobin. Moreover, the results of the present investigation with regard to oxygen capacity in anaemias including haemoglobinopathies (i.e., a reduction of the capacity in whole blood and its subsequent increase in haemoglobin solutions) proved that the stroma of erythrocytes in anaemia plays quite an important role in the oxygenation of haemoglobin; and it is more evident in red cells with abnormal haemoglobins.
The above information prompted us to carry on investigations on red cell membrane or stroma in cases of haemoglobinopathies.

The stroma is composed chiefly of protein and lipids. The present investigations attempted to assess the structural condition of the stroma in haemoglobinopathies. With this in view total lipids were determined. Estimation of total nitrogen and hexosamine were also done to get information about total protein and mucopolysaccharide-content of the stroma.

In the present series of five normal subjects the average value of total lipids of the stroma was 13.5%. Bernstein et al (1938) by studying three samples of blood from normal subjects found the total lipid content of the stroma to be 10-12%. They developed a procedure of yielding stroma which was based upon haemolysis of the erythrocytes and prolonged washing of the stroma with large volumes of sodium citrate buffer, pH 5.5.

In the present investigation the values of total lipids in stroma showed a definite increase in haemoglobinopathies studied than those in normal subjects. The value was more in Hb E-thalassaemia disease than in thalassaemia major group of patients. Total lipids in stroma went up to 20% in the average in Hb E-thalassaemia disease. The total lipid content could not be correlated with the percentage of Hb F in haemoglobinopathies studied. Thus R.G. (Serial No. 39) had 10.5% of foetal haemoglobin and his stroma contained 18% of total lipids, whereas S.P. (Serial No.36) with 19.8% foetal haemoglobin had 19.5% stroma-lipid.

A few workers estimated lipid content of the stroma. Erickson et al (1937) studied the red cell lipid in different anaemias. Schwarz-Tiens et al (1955),
Sarkar (1961), De Gier and Van Deenen (1964) and Nicolopoulos et al. (1966) — all of them studied the total lipid and the proportions of individual types of phospholipids of erythrocytes obtained from thalassaemic and other anaemic patients. Phospholipid content was found to be increased in thalassaemias. These workers undertook different methods of investigation. Some resorted to chemical determination while others conducted chromatographic studies.

Fels et al. (1961) have shown elevated red cell cholesterol in sickle cell anaemia accompanied by slightly subnormal plasma levels. They have put forward a probable explanation that the life of the red cell is conditioned by the lipid-cholesterol concentration of its surface. When a certain concentration is achieved the red cell is susceptible to capture by the R.E. cells where it is destroyed. They have also predicted that in hypocholesterolaemia it is shortened. So there is a possibility that the high total lipid content in stroma of haemoglobinopathies in the present study may cause a greater sensitivity to lytic action of the reticuloendothelial system, thus resulting in a shortened life span of these erythrocytes.

Mucopolysaccharides occur in epithelial and connective tissue. The most important member of this group is hyaluronic acid. The levels of mucopolysaccharides have most frequently been determined and expressed in terms of hexose or hexosamine content.

The hexosamine content of the stroma in normal subjects in the present series was 1.51% in the average, when expressed as hexosamine HCl. Indewig (1960) has reported the hexosamine content of the stroma in normal subjects to be 1.1 to 1.3%, expressed as hexosamine HCl. Indewig subjected the acid
hydrolysate of the stroma through a column of Dowex 50 before estimating the hexosamine content. In this way he could get rid of the amino acids that might have contaminated the hydrolysate. This can explain a relatively higher value of hexosamine in the present series. In haemoglobinopathies however the values showed a slight increase. The average values were: in thalassaemia major, 1.81% and in Hb E-thalassaemia disease, 1.75%. But when all these values of hexosamine were expressed in terms of total nitrogen no relative increase could be elicited. The range of hexosamine content of the stroma in normal subjects as well as in haemoglobinopathies varied between 0.12 and 0.16 mg. when expressed in terms of 1 mg. of nitrogen. In one case of thalassaemia major (Serial No. 22) the value was 0.17 mg. as per mg. of nitrogen.

From the results of study of hexosamine content of stroma it can be presumed that the mucopolysaccharides of the stroma are not disturbed in haemoglobinopathies.

The total nitrogen contents of stroma in normal subjects and in haemoglobinopathies in the present investigation did not vary much. The average values were: in normal subjects, 10.66%, in thalassaemia major, 11.28% and in Hb E-thalassaemia disease, 11.18%. Beach et al. (1939) investigating three preparations of stroma for total nitrogen have shown the value of 13.00% in the average.

The value of total nitrogen of stroma 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. From these investigations it may be presumed that the total protein content of stroma does not undergo much change in haemoglobinopathies.

From the study of the stroma it could be observed that the mucopolysaccharides and the total protein did not undergo any gross change in haemoglobinopathies. The only component of stroma which showed a high value in haemoglobinopathies was total lipid.

There seems to be a great possibility of linking the findings of oxygen capacity with those of erythrocyte stroma in haemoglobinopathies studied in the present series. The reduction in oxygen capacity in anaemias, and particularly in haemoglobinopathies, \textit{in vitro}, indicates the interference by the red cell membrane to the passage of oxygen into the cell for combination with haemoglobin. The degree of reduction in various groups of cases investigated may vary well indicate the degree of interference by the cell membrane.

The particular factor responsible for the interference is yet to be known. In the present investigation the high content of total lipids in erythrocyte stroma of haemoglobinopathies may be suggested as being partially responsible for the interference in the entry of oxygen into the cell. There may be some sort of thickening of the cell membrane, and also associated physico-chemical change due to high lipid content so that the permeability of the cell membrane to gases is reduced. When this membrane was taken off, \textit{i.e.}, when oxygen capacity was determined in haemoglobin solutions, the capacity was found to be increased.

That the capacity-increase in haemoglobin solutions was different in
different groups of investigation may be accounted for with the assumption that individual haemoglobin was peculiar with respect to the acceptance of oxygen. It also depended upon whether the normal adult haemoglobin in a particular case was partially or wholly replaced by abnormal haemoglobins. Reports on relative values of oxygen capacity in various haemoglobinopathies are lacking in literature. From the present series it can be stated that the capacity in haemoglobin solution containing mostly abnormal haemoglobins, and practically no adult haemoglobin showed a lesser increase than in haemoglobin solution containing mixtures of abnormal haemoglobins with adult haemoglobin. Moreover, the results of study of the lipid content of haemoglobin solutions show that the content of total lipids is comparatively higher in solutions of abnormal haemoglobins than that of adult haemoglobin. This may be an added factor for the lesser increase of oxygen combining capacity in solution of abnormal haemoglobins than the solution containing mixtures of abnormal haemoglobin with Hb A.