SUMMARY

1. A comparative study of haemoglobins of two mammalian groups namely, primates and ruminants and one of avians was undertaken to evaluate:
   (a) the structural basis for the heterogeneity, and
   (b) the genetic basis for haemoglobin phenotypes.
In primates the comparative study was restricted to the haemoglobins of human, langurs and bonnets. Water-buffalo, bovine and Indian breeds of sheep and goat were studied from ruminant group. In case of avians, chick, duck and pigeon each of which represents a separate natural order, were taken for the study of their haemoglobin types.

2. Prior to haemolysis, the haematological data and the fragility of erythrocytes in percent of saline were determined. The haematological values of human, langur and bonnet closely resembled each other. In case of ruminants, water-buffalo and bovine were having nearly similar haematological values but those of sheep and goat were quite different. Avians also resembled each other in their haematological values.

Osmotic fragilities of erythrocytes of human, langur and bonnet were found to be similar. In case of ruminants, red cells were more fragile than those of
primates and avians. In avians all the three species showed identical erythrocyte osmotic fragility.

3. A systematic survey of haemoglobin types from each species was carried out by paper and starch gel electrophoresis using different buffer systems and by CM-cellulose column chromatography.

4. Langurs showed multiple haemoglobins, involving either two components together or one component singly corresponding to the faster type. Likewise, in case of bonnet haemoglobins there seemed to be a similar pattern of occurrence. Among ruminants bovine and sheep haemoglobins exhibited polymorphism involving two haemoglobins while buffalo haemoglobins were found to be monomorphic consisting of two components. Multiple haemoglobins of goat showed either two components together or one component singly corresponding to the faster type. This situation is comparable to that of langur as described above. A third haemoglobin, goat Hb-3, was also encountered during our survey. The haemoglobins of langur, bonnet and buffalo did not resolve into two components on paper electrophoresis. Hence CM-cellulose column chromatography proved to be the only method to separate the two components of haemoglobins in each of the species.

5. On paper electrophoresis, mobilities of human, langur,
bonnet and buffalo haemoglobins were identical. The maximum net charge difference was observed in case of bovine haemoglobins. Avian haemoglobins, in general, showed less anodic mobility and revealed the presence of a minor component along with a major one.

6. In primates, the haemoglobins underwent immediate denaturation by alkali. Ruminant haemoglobins, on the other hand, were highly resistant to the denaturation. Among the avians, the duck haemoglobin is more resistant to denaturation by alkali than that of chick and pigeon.

7. The nature of the polypeptide chains of various haemoglobin types was studied by paper electrophoresis using urea-veronal buffer. The haemoglobins of langur and bonnet had α and β polypeptide chains of similar mobility. The mobilities of these α and β subunits were also comparable to those of human haemoglobin.

The buffalo haemoglobins had α-chains of different mobility while the β-chains appeared similar. The haemoglobins of bovine and sheep within species have α-chains of identical mobility, while the mobilities of β-chains were different. The two goat haemoglobins, Hb-1 and Hb-2 had α-chains of different mobility while the mobilities of β-chains are similar.

In case of avians, starch gel electrophoresis using
Nass-formate buffer was carried out to evaluate the nature of the polypeptide chains. The $\alpha$ and $\beta$ subunits of chick major and minor haemoglobins exhibited different mobilities. Chick major, duck major and pigeon haemoglobins had $\alpha$ and $\beta$-chains of comparable mobility. 8. The separation and isolation of polypeptide chains were achieved by CM-cellulose column chromatography using Na$^+$ gradient. The globins and the polypeptide chains (wherever necessary) were amino-ethylated and characterized by tryptic peptide pattern analysis.

9. Fingerprints of langur Hb-1 and langur Hb-2 were indistinguishable. Similar was the case with bonnet haemoglobins. The interspecies comparison of human, langur and bonnet revealed a specific difference in the position of one tryptic peptide containing tryptophane. This differing tryptic peptide is a $\beta$ Tp-II one.

Examination of the fingerprints of the $\alpha$ and $\beta$ subunits of langur and bonnet haemoglobins revealed that within species the $\alpha$-chains were similar while the $\beta$-chains were different. Furthermore, the $\alpha$ and $\beta$ subunits of the haemoglobins of human, langur and bonnet species were largely similar, except for a few differing peptides.

The fingerprint of buffalo Hb-1 differed from that of buffalo Hb-2 in two tryptic peptides one of which contains tryptophane. These differences were found to be
restricted to α-chains while β-chains appeared to be similar. In bovines and sheep the two polymorphic haemoglobins within species shared a common α-chain while multiple differences existed in β-chains. This has been substantiated recently. The two goat haemoglobins differed in α-chains and had common β-chains. The third goat haemoglobin, Hb-3 shared a common α-chain with that of Hb-2, while non-α-chains were different. Interspecies comparison of the homologous polypeptide chains of buffalo and bovine indicated that the respective α and β-chains of these two species were different. In case of sheep and goat, on the other hand, it appeared that the α-chains of sheep haemoglobins and that of goat Hb-2 might be identical.

In case of avians, the fingerprints of chick major and chick minor haemoglobins revealed many tryptic peptide differences. Likewise, multiple differences existed in the haemoglobins of duck major and minor. The interspecies comparison of chick major, duck major and pigeon haemoglobins revealed that all the three haemoglobins were quite different from one another.

10. Based on these structural studies, the genetic basis for the haemoglobin types in primates, ruminants and avians is discussed. Finally the evolution of haemoglobin is discussed in general.