Fetal and Embryonic Haemoglobins

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Haemoglobin has been the subject of intensive research for many years and is one of the most thoroughly understood of all protein molecules. The amino-acid sequences of haemoglobins from many species of animals have been determined (tabulated by Dayhoff, 1969) and the molecular structures of horse and human haemoglobins have been determined in great detail by x-ray crystallography (Perutz et al, 1968a and b; Perutz 1969). A mechanism of action of haemoglobin has been proposed (Perutz, 1970a and b and 1972). The haemoglobins of higher organisms share a common tetrameric structure built up of two pairs of unlike chains; the α chains containing 141 amino-acid residues and the non-α chains containing generally 145 or 146 amino acids. In man, five types of non-α chains have been recognized, β, γ, δ, ε, and ζ, corresponding to different stages of ontogenesis. The globin chains of haemoglobin and of myoglobin have a similar overall tertiary structure composed of segments of α helix interspersed with non-helical regions forming a compact structure of great complexity. Amino-acid residues whose side chains are of a polar nature, ie, those which bear an electric charge or a strong dipole movement are located at the outer surface of the molecule in contact with the aqueous surroundings, whereas the non-polar hydrocarbon side chains are, in general, located within the interior of the molecule. The haem groups are accommodated within deep cavities or pockets lined with non-polar side chains and are anchored in position by links between the haem iron and the imidazole side chains of histidines. The non-aqueous environment of the haem promotes reversible binding of oxygen and protects the iron from oxidation to the ferric form characteristic of methaemoglobin which is incapable of reversible oxygen binding.

The four chains of the tetramer fit together to form a nearly spherical molecule with extensive areas of contact between unlike chains; the two main types of contact are denoted αβ1 and αβ2. The tetramer exhibits cooperative behaviour or haem–haem interaction. As each haem combines with oxygen the affinity of successive haems increases. The oxygen affinity curve of the tetramer is sigmoidal and may be represented approximately by the Hill equation:*  

\[ y = \frac{kp_{o2}^n}{1 + kp_{o2}^n}. \]

Oxygen affinity data are usually presented in terms of \( P_o2 \), the partial pressure of oxygen required to attain half saturation with oxygen, and of \( n \), the exponent of the Hill equation. These parameters respectively give measures of the oxygen affinity and of the degree of haem–haem interaction. For normal haemoglobin \( n \) is approximately 3. Myoglobin, isolated chains, and tetramers composed of one type of chain such as Hb H (β4) show no haem–haem interaction, their oxygen dissociation curves are hyperbolic and the \( n \) value is 1.

The haemoglobin tetramer has a lower oxygen affinity, more appropriate of its physiological function, than single chains. The cooperative behaviour of the tetramer renders it more efficient in releasing oxygen in response to relatively small changes in oxygen tension. The tetramer exhibits other physiologically advantageous properties; the oxygen affinity varies with \( pH \) (Bohr effect) in such a manner as to facilitate the release of oxygen in the tissues. Haemoglobin binds CO at the N terminus of the β chains (Kilmartin and Rossi-Bernardi, 1969) and assists its transport from the tissues to the lungs. Furthermore its oxygen affinity is reduced in the presence of organic phosphates particularly 2,3 diphosphoglycerate (2,3 DPG) and inositol.

* Hill equation: \( y = \frac{kp_{o2}^n}{1 + kp_{o2}^n} \) partial pressure of oxygen, \( k \) and \( n \) are constants.
hexaphosphate (IHP) (Benesch and Benesch, 1969). This provides an important method of regulating the oxygen affinity in vivo. The properties can be accounted for in terms of the molecular structure (Perutz, 1970a and b).

Many species of animals contain multiple haemoglobin variants which can be separated by electrophoresis. The components are often present in unequal proportions eg, in man Hb A and Hb A2 are present in the ratio of about 40:1. Some of the components may be chemically modified forms of other components; for example the minor component Hb A1c of human blood consists of Hb A modified by attachment of a carbohydrate residue at the N terminus of the β chain (Bookchin and Gallop, 1968). Other multiple components represent the products of separate gene loci and contain globin chains which differ by one or more amino-acid substitutions, thus the β and δ chains of human Hb A and Hb A2 differ by 10 amino-acid substitutions.

Fingerprinting has revealed differences between the haemoglobins of different species (Muller, 1961) and in many cases the exact amino-acid sequences have been determined (Dayhoff, 1969). In general each species has one or more haemoglobin components unique to itself. The more closely related two species are, the more similar are their haemoglobins. In man and chimpanzee the major adult haemoglobin Hb A is identical and the minor component Hb A2 differs by only one amino-acid substitution (de Jong, 1971). In various species of the genus Equus haemoglobin components differ by one amino-acid substitution (Kitchen and Easley, 1969).

When the amino-acid sequences of the globin chains of haemoglobins and myoglobins from many species are compared they show a great degree of homology if gaps are introduced at the appropriate positions (Braunitzer et al, 1964).

Such comparisons of globins and other proteins, especially cytochrome c (Dayhoff, 1969), have provided a novel basis for the construction of phylogenetic trees based on data quite independent of conventional zoological characteristics. Furthermore these comparisons have lent support to the speculation that the haemoglobin and myoglobin chains may themselves have diverged and evolved from a more primitive 'ancestral molecule'. The general manner in which this may have come about has been outlined by Ingram (1961), who postulated that an initial single gene locus specifying a globin chain may have undergone duplication followed by a gradual divergence of the two genes by independent mutations and deletions. Successive duplications may have given rise to the genes for the families of polypeptide chains observed in each species. It is postulated that a gradual divergence of the structure of the polypeptide chains led to their ability to associate with each other to form dimers and tetramers and the appearance of haem–haem interaction and the Bohr effect. It seems likely that the 'ancestral molecule' was probably longer than modern globin chains because of the need to postulate deletions and the fact that the globin chains of more primitive animals eg, lamprey are longer than those of higher animals. It is a matter of controversy whether the differences in amino-acid sequences of haemoglobin chains of different animals may be attributed to the action of Darwinian natural selection or to the action of genetic drift and the fixation in different species of amino-acid substitutions which cause no significant change in the properties of the haemoglobin and are selectively neutral (review by Harris, 1971). Some parts of the amino-acid sequence of the globin chain show much less interspecies variability than others and some residues are invariant in all species. In general, the amino acids whose side chains form the lining of the haem pocket and the surfaces of the α1β2 interface show very little variability and any substitutions are of a conservative nature ie, one amino acid is replaced by another of a similar character. In contrast, amino acids located at the outer surface of the molecule show wide variability.

In studying the haemoglobins of animals it is practicable to examine the specimens from only a small number of animals. The situation is different with the human population where blood samples from many people have been examined by electrophoresis during the course of routine haematological examinations and surveys.

These studies have given a measure of the degree of intraspecific variation of haemoglobin in the human population and have led to the discovery of over 160 different mutant forms of haemoglobin (reviewed by Huehns and Shooter, 1965; Lehmann and Carrell, 1969; Lehmann, 1972). The study of human haemoglobin mutants has afforded much valuable information. Studies of the inheritance of abnormal haemoglobins showed that the α and β chains were specified by separate gene loci, and this is also true for γ and δ chains and presumably also for the ε and ζ chains. Some recent evidence (discussed below) suggests that, at least in some individuals, the α-chain locus is duplicated. The γ-chain locus is also duplicated and the polypeptide chains specified by different loci differ by at least one amino-acid substitution (Schroeder et al, 1968).

The study of rare pathological haemoglobins in which the physiological functions are impaired has played an important role in the understanding of
the normal functioning of haemoglobin (Perutz and Lehnmann, 1968). Many of these haemoglobins have mutations at sites which are normally invariant.

Despite great advances in our understanding of the structure and function of haemoglobins many problems remain to be solved. The factors which regulate the rate of production of α and non-α chains and ensure that they are produced in equivalent amounts are not understood. In the thalassaemias there is an imbalance of chain production which results in the formation of one or other type of chain in excess with consequent disturbance of function (Weatherall and Clegg, 1972). The exact nature of the lesion in the thalassaemias is not yet known (Weatherall, 1968). It is not known why multiple haemoglobin components which are the products of separate genetic loci are not necessarily produced in equal amounts (eg, Hb A and Hb A2) nor why there are changes in the proportions of different components during development or under conditions of stress. The discovery that haemoglobin messenger RNA is longer than is required to code for the structure protein and contains additional sequences relatively rich in adenylic acid (Lim and Canellakis, 1970) may have some bearing on these questions. Possibly the untranslated regions may be involved in the regulation of protein synthesis.

Another fascinating problem is that of the changes of haemoglobin type which occur during fetal and embryonic development and the changes which occur in some adult animals, in particular sheep and goats, on exposure to the stress of anaemia. Although the haemoglobin types involved have been partly characterized in some cases, very little is known of the factors which control these developmental changes. An understanding of these factors would be of both fundamental, theoretical, and also of practical interest as a possible basis of therapy for some haemoglobinopathies.

Fetal and Embryonic Haemoglobins

Ontogeny of Haemoglobin. In several species of animals it has been observed that there is a change in the type of haemoglobin during embryological and fetal development. In man, early embryos contain two embryonic haemoglobins; these are replaced successively by fetal haemoglobin Hb Fα2γ2 and finally by the adult haemoglobins Hb A and Hb A2 beginning before birth (Huehns and Shooter, 1965). The amount of Hb F present at birth is generally about 70–90% of the total haemoglobin. This declines during the first 12 months after birth; less than 8% is present at 6 months and less than 2% at 12 months (summarized by Huehns and Beaven, 1971). It has been suggested that these different haemoglobins represent adaptations to different modes of obtaining oxygen (Huehns and Beaven, 1971). In the early embryo, oxygen is obtained from the maternal interstitial fluid, in the developing fetus it is obtained via the placenta, and after birth via the lungs. It is, however, possible for a fetus to survive with adult blood after an exchange transfusion (Dawes, 1967), and for certain rare adult individuals to survive although their red cells contain only the fetal type of haemoglobin.

Embryonic Haemoglobins. The first definite evidence for the existence of distinct embryonic haemoglobins in man was reported by Huehns et al. (1961) who found two new haemoglobins in a small embryo of 3.5 cm crown-rump length (CRL) from a pregnancy of 9 weeks duration. No haemoglobins different from Hb A and Hb F were found in 25 embryos from pregnancies of 10 weeks or more. The two haemoglobins, which were called Hb Gower 1 and Hb Gower 2, migrated much more slowly than Hb A on starch gel electrophoresis at pH 8.6; Hb Gower 1 was the faster, migrating just behind Hb A2. In a further study of haemoglobins from 12 embryos Huehns et al (1964b and c) found Hbs Gower 1 and 2 to be present in embryos of CRL up to 6 cm; only traces of Hb Gower 1 were present in embryos up to 8.5 cm CRL and none was detected in larger embryos. In the smallest embryo examined, CRL 2.5 cm, there was 24% Hb Gower 1 and 13% Hb Gower 2. Small amounts of Hb Gower 2 were found in one out of 300 cord blood samples. Other workers have reported similar findings, eg, Horton et al (1962) and Schneider et al (1970) who found one case of Hb Gower 2 in a survey of over 11,000 cord blood samples. Hb Gower 2 has also been found in small amounts (0.1–0.7%) in red cells of newborns with the D1 trisomy abnormality. Examination of Hb Gower 2 by fingerprinting (Huehns et al, 1964c) indicated that it is composed of α chains and another type of chain, denoted ε, which differs from both the β and γ chains. Hb Gower 2 probably has the structure α2ε2. Hybridization studies indicated that the Hb Gower 1 probably consists entirely of ε chains ie, Hbe4. The ε chains must be considerably more basic than the β or γ chains as Hb Gower 2 differs from Hb A by about 6 units of positive charge.

Other mammals show a similar development of specific embryonic and fetal haemoglobins. Kleihauer, Brauchle, and Brandt (1966) examined haemoglobins from embryos of Simmentaler cattle of 0.5–6.0 cm CRL. The haemoglobins were
separated by starch gel electrophoresis. Embryos of 3-0-7-0 CRL had fetal haemoglobin and two embryonic haemoglobins which migrated slowly towards the anode on electrophoresis at pH 8-6. Smaller embryos (1-0-1-5 cm) sometimes had three which were denoted 1, 2, and 3 in order of decreasing anodal mobility; fraction 1 had the same mobility as human Hb A2. No fetal haemoglobin was detected in the smallest embryo (0-5 cm) which contained only fractions 2 (90%) and 3 (10%). In all cases fraction 2 was present in greater amounts than fractions 1 and 3. Embryos of 3-0 cm and longer had only fractions 1 and 2. In embryos of 6-0-7-0 cm the embryonic haemoglobins amounted to only 2% of the total haemoglobin and only traces were detected in larger embryos. Kleihauer and Stöffler (1968) detected two haemoglobin fractions analogous to Hbs Gower 1 and 2 in early embryos of cattle, sheep, and pig; these fractions were gradually replaced by fetal haemoglobins. Embryonic haemoglobins have been found in chickens (Manwell et al, 1963) and ducks (Borgese and Bertles, 1965). Changes in haemoglobins have also been observed during the development of the adult frog R. catesbiana from the tadpole (Moss and Ingram, 1968a and b).

It is not certain whether Hb A is present in early human embryos. A protein which migrated in the position of Hb A at pH 8-6 was found to have different mobility from Hb A at pH 6-2 (Kaltsoya, Fessas, and Stavropoulos, 1966). This component may be identical with Hb Portland 1 (Capp, Rigas, and Jones, 1967) which is thought to be a tetramer composed of γ chains and a fifth type of chain called the ζ chain i.e., γζζζ (Capp, Rigas, and Jones, 1970). The ζ chain has been found in cord bloods from several normal and abnormal infants (Todd et al, 1970).

Fetal Haemoglobin. In 1866, Körber discovered that blood obtained from the human placenta was more resistant to denaturation by acids and alkalis than normal blood. This discovery was the first indication that there was a difference between the haemoglobins of adults and of fetuses and newborns. The early history of this work has been reviewed by Bertles (1969). Human fetal haemoglobin Hb F differs from adult haemoglobin Hb A in several physical properties. It is much more resistant to denaturation in alkaline solution and this property provides the simplest way of detecting and assaying it (Singer, Chernoff, and Singer, 1951). Hb F and Hb A can be distinguished immunologically (Darrow, Nowakovsky, and Austin, 1940). Hb F has a slightly different ultra-violet spectrum from Hb A; it shows a fine structure band at 289-8 nm which is absent from Hb A (Beaven, Hoch, and Hobday, 1951); the difference is due to the higher content of tryptophan in Hb F. Both glycine and valine are present as N terminal amino-acid residues in Hb F; only valine is found at the N terminus of Hb A; isoleucine is present in Hb F and absent from Hb A.

Structure of Hb F. Haemoglobin F is a tetrameric molecule composed of a pair of α chains identical to those in Hb A and Hb A2 (Schroeder et al, 1963b) and a pair of γ chains. The γ chains contain 146 amino acids with the sequence shown in Fig. 1; this differs from that of the β chains in 39 positions as indicated (Schroeder et al, 1963a).

Although the three-dimensional structure of Hb F has not been determined in detail it is likely that it is similar to that of adult haemoglobin. Approximately two thirds of the amino-acid substitutions in the γ chain are on the external surface and are likely to have relatively little effect on the physiological properties of the molecule. There are nine changes of internal residues including the exchange of tyrosine 130 for tryptophan which is responsible for the different ultra-violet spectrum of Hb F. These substitutions are mainly of a conservative nature in which one amino acid is replaced by another of a similar type. Four substitutions occur at the α1β1 interface. These may account for the reduced tendency of Hb F to dissociate into monomers which is the probable cause of its resistance to alkaline denaturation (Baglioni, 1963; Huehns et al, 1964a). The residues at the α1β2 interface are unchanged.

An important difference between Hb F and Hb A arises from the substitution of histidine β 143 by serine in the γ chain. Perutz (1970a and b) has suggested that 2,3, DPG binds to deoxy haemoglobin in a cavity between the β chains lined with positively charged groups, the α chains of the N terminal residues, the ε amino groups of lysine 82, and the imidazole group of histidine 143; this has recently been confirmed by x-ray crystallography (Arnone, 1972). DPG stabilizes the deoxy form of haemoglobin which results in a reduced oxygen affinity. In Hb F the replacement of histidine by serine removes two positively charged residues from the cavity resulting in DPG being less strongly bound. Consequently the oxygen affinity of Hb F is not reduced by DPG so much as that of Hb A.

Haemoglobin F Variants. Extensive surveys of thousands of cord blood samples have been carried out and a number of fetal haemoglobin variants
have been found. Both $\alpha$- and $\gamma$- chain variants have been detected. When the mutation is in the $\alpha$ chain an abnormal haemoglobin is also present during adult life, but when the mutation is in the $\gamma$ chain an abnormal haemoglobin is present only during fetal life and for a few months after birth. The known $\gamma$-chain variants are listed in Table I; they all appear to be harmless. In some cases the amino acid present at position $\gamma$ 136 has also been identified.

### TABLE I

<table>
<thead>
<tr>
<th>Name</th>
<th>Substitution</th>
<th>136</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>F Texas II</td>
<td>6 A3 Glu Lys</td>
<td>?</td>
<td>Larkin et al (1968)</td>
</tr>
<tr>
<td>F Malta</td>
<td>117 G19 His Arg</td>
<td>Al</td>
<td>Cauchet et al (1969)</td>
</tr>
<tr>
<td>F Malia</td>
<td>?</td>
<td>Al</td>
<td>Huisman et al (1972)</td>
</tr>
<tr>
<td>F Hull</td>
<td>12 GH4 Glu Lys</td>
<td>(Ala)*</td>
<td>Larkin et al (1967)</td>
</tr>
<tr>
<td>F Roma</td>
<td>?</td>
<td>?</td>
<td>Silverstroni and Bianco (1963)</td>
</tr>
<tr>
<td>F Dickenson</td>
<td>97 FG4 His Arg</td>
<td>?</td>
<td>Schneider et al (1970)</td>
</tr>
<tr>
<td>F Kuala Lumpur</td>
<td>22 B4 Asp Gly</td>
<td>Ala</td>
<td>Larkino et al (1973)</td>
</tr>
</tbody>
</table>


**Haemoglobin Barts $\gamma_4$.** Haemoglobin Barts (Ager and Lehmann, 1968) is a tetramer composed of four normal $\gamma$ chains and is analogous to Hb H ($\beta_4$). On electrophoresis at pH 8.6 it migrates more rapidly towards the anode than Hb A and for this reason was formerly called 'fast fetal haemoglobin' (Fessas and Papaspyrou, 1957). It may be isolated by chromatography on IRC-50 ion exchange resin. Hb Barts shows a more well resolved tryptophan fine structure band at 289-6 nm than Hb F; it is more resistant to alkaline denaturation than Hb A but less so than Hb F (Dance, Huehns, and Beaven, 1963). Haemoglobin Barts has a very high oxygen affinity and shows no haem–haem interaction or Bohr effect (Horton et al, 1962); it is physiologically useless haemoglobin as it is unable to release its oxygen to tissues.

Small amounts of Hb Barts have been detected in normal cord bloods by starch gel electrophoresis and staining for haem protein with benzidine (Fessas and Mastrokalos, 1959); it is also found in early fetal and embryonic life. Increased amounts of Hb Barts are found in the cord bloods of individuals suffering from thalassaemia. In the $\alpha_1$ thalassaemia trait there is about 5–10% Hb Barts (Na-Nakorn et al, 1969). In the homozygous condition there is complete suppression of $\alpha$-chain synthesis and the fetal cells contain only Hb Barts. Death in utero results from the failure of the haemoglobin to release its oxygen (Lie Injo, 1962).

**Fetal Haemoglobins in Other Animals.** Many species of mammals and other animals have been examined for the presence of fetal haemoglobins. It has been found that resistance to alkaline denaturation is not a universal property of fetal haemoglobin. Beaven and Gratzier (1959) detected a fetal haemoglobin in the rhesus monkey, *Macaca mulatta*, by agar gel electrophoresis. The fetal haemoglobin migrated more slowly than the adult haemoglobin in this system, the order of migration being the opposite to that in humans. *M. mulatta* fetal haemoglobin showed a tryptophan fine structure band at 289-7 nm and was resistant to alkaline denaturation. Sen, Das, and Aikat (1960) measured the rate of disappearance of alkali resistant haemoglobin in infant *M. mulatta*. The amount of alkali resistant haemoglobin averaged 48.9% in four new born monkeys and fell to the adult level (average 2.9% in 60–90 days; only one component was detected in both adult and fetal haemoglobin. Other species of Macaca show interesting differences.

Kitchen, Eaton, and Stenger (1968) examined haemoglobins from fetal and adult *Macaca speciosa* (stump-tailed macaque) by starch gel electrophoresis and peptide mapping. Two adult and two fetal haemoglobin components were detected. In adults the electrophoretically faster component represented 60–65% of the total and the slower component 34–40% although this ratio was reversed in some animals. The two components differed in their $\alpha$ chains, a difference in peptide $\alpha$ Tp 3 was detected by peptide mapping. No embryonic haemoglobins were detected, but embryos younger than 47 days gestation were not examined.

In contrast to the situation in *M. speciosa*, Nute and Stamatoyannopoulos (1971) found evidence for two types of chain in *Macaca nemestrina* (pig-tailed macaque). They examined haemolysates from fetal and infant macaques ranging from 60 days gestation to 86 days after birth (gestation period 170 days). Haemolysates from fetuses contained either two (in the younger ones) or three components. The third component had the same electrophoretic mobility as the single band found in adult animals. The two fetal haemoglobins, F slow and F fast, were present in the ratio of approximately 2:1. The proportions in seven fetuses between 132 and 167 days gestation estimated by DEAE Sephadex chromatography were F slow 63–68%,
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F fast 30–35%, and A 0–5%. Fingerprinting of the isolated α and γ chains indicated that the α chains were probably identical but that there were several differences between the γ chains. The γ chain of the fast component appeared to have a lower content of tryptophan.

It is possible that *M. speciosa* may contain two types of γ chain which are electrophoretically indistinguishable as is the case in man and also in the chimpanzee (de Jong, 1971). In view of the discovery that the ratio of the two types of γ chains in humans changes after birth, it would be interesting to know whether this also occurs in *M. nemestrina*; technically this would be easier because it is possible to separate the two components by electrophoresis.

Alkali resistance is not a property specific to fetal haemoglobins. Buettner-Janusch and Twichell (1961) examined several species of prosimian primates for alkali resistant haemoglobins. High levels of alkali resistant haemoglobins (40–80%) were found in adult individuals from several species of Lemuriformes and Lorisiformes (10–60%).

Adults from several species of anthropoid apes showed, in general, low levels of alkali resistant haemoglobins. High levels of alkali resistant haemoglobin were found in infant and fetal *Macaca mulatta* and in infant *Cebus apella*.

Fetal haemoglobins have been detected in some common domestic mammals and other animals. Kleihauer et al (1966) and Kleihauer and Stöffler (1968) demonstrated that cattle, sheep, and pig embryos all go through a type of development similar to human embryos first producing two embryonic haemoglobins analogous to human Hbs Gower 1 and 2 which are later replaced by a fetal fraction and finally by the adult form. The amino-acid sequence of the γ chain of bovine fetal haemoglobin has been determined (Babin et al, 1963). It contains 145 amino acids, one fewer than in human γ chain. It has N terminal methionine and differs from the human β chain in 32 positions and from the human γ chain in 40 positions. A nearly complete sequence for the γ chain of sheep Hb F was obtained by Wilson et al (1966). Sheep of genotypes AA, BB, and AB, which share a common α chain, have the same type of fetal haemoglobin. In goats, two types of fetal haemoglobins are found according to the genotype of the goat. These haemoglobins have a common γ chain and differ in their α chains. This variation reflects a similar variation in the adult haemoglobin types. The rather complex pattern of haemoglobins in the goat has been summarized by Huisman et al (1969a).

In fetal mice of strain C57/BL 6J there are two distinct populations of erythrocytes. One population is composed of nucleated cells probably derived from the yolk sac blood islands; the second population is composed of smaller non-nucleated cells from the fetal liver and developing bone marrow (Craig and Russell, 1964). The first population contained at least three haem proteins and the second contained a single haemoglobin different from those in the first. The haemoglobins (E1, E11, and E11) from the yolk sac cells were separated into their constituent chains which were of five types denoted x, y, z, α, and β (Fantoni, Bank, and Marks, 1967) Hb E1 contained x and y chains; E11 contained α and γ chains, and Hb E11 contained x and z chains. The haemoglobin from the liver-derived cells was indistinguishable from the adult haemoglobin composed of α and β chains. The Hbs E11 and E11 differed only in the nature of their non-α chains. It was pointed out that one of the embryonic chains might differ from another only by the presence of an N terminal acetyl group, as the case in human Hb F (Schroeder et al, 1962) and in bullfrog haemoglobin (de Witt and Ingram, 1967). Possibly the two erythroid cell lines may develop from different precursor cell lines in the respective tissues or alternatively the erythroid cells of the yolk sac blood islands may seed the developing liver and change the types of haemoglobin produced in response to the new environment.

**Detection and Isolation of Fetal Haemoglobin.** The most common method of detecting and estimating Hb F is based on its resistance to alkaline denaturation. In the method devised by Singer et al (1951) a dilute haemolysate is exposed to NaOH solution at pH 12.7 for 1 minute, and the solution is then neutralized. Oxyhaemoglobin A is denatured by this treatment and is precipitated with ammonium sulphate; Hb F remains in solution and is estimated spectrophotometrically. The method has the advantage of simplicity but tends to give imprecise results at low levels of Hb and results that are too low with high levels of Hb F (Jonxis and Huisman, 1956). More precise estimates may be obtained by measuring the actual rate of denaturation of the sample under standard conditions (reviewed by Huehns and Beaver, 1971). The method has been adapted to a microscale for studying small samples from embryos.

A modification of the Singer method using cyanmethaemoglobin for measuring samples containing less than 10% Hb F has been described by Betke, Marti, and Schlicht (1959).

Intracellular Hb F can be detected by the Kleihauer test (Kleihauer, Brown, and Betke, 1957).
which is based on the observation that Hb F is less readily eluted than Hb A from cells in citrate buffer, pH 3.2. After elution of the haemoglobin the cells are stained with haematoxylin and eosin. The degree of staining is proportional to the amount of Hb F in the cells. Cells which contained only Hb A are visible as 'ghosts' while cells containing large amounts of Hb F, eg, cord cells are heavily stained.

The cellular distribution of Hb F may also be determined by using a fluorescent-labelled antibody against Hb F (Tomoda, 1964; Hosoi, 1965; Dan and Hagiwara, 1967). Both these methods illustrate that individual erythrocytes are capable of synthesising both Hb A and Hb F.

**Isolation of Hb F.** Human Hb F is not readily separated from Hb A by paper electrophoresis. Satisfactory separations for diagnostic purposes may be achieved by electrophoresis in agar gel or starch gel (Huehns, 1968; Huehns and Beaven, 1971).

Haemoglobin F may be separated best by ion exchange chromatography. Prins and Huisman (1955) separated Hbs A, S, C, and F on IRC-50, a cation exchange resin using sodium citrate buffers. Allen, Schroeder, and Balog (1958), using a phosphate buffer containing cyanide, obtained a good separation of Hb F from Hb A, and observed that Hb F from cord blood splits into two fractions F₁ (20%) and F₂ (80%). These two components are equally resistant to alkaline denaturation (Matsuda et al, 1969), and differ only in that the N terminal residue of the γ chain in Hb F is acetylated.

Acetylated N terminal residues have been found in haemoglobins of other animals, and an enzyme has been found which acetylates the N terminus of chicken haemoglobin (Marchis-Mouren and Lipmann, 1965).

Haemoglobin F may also be separated by chromatography on DEAE Sephadex (Huisman and Dozy, 1965; Dozy, Kleihauer, and Huisman, 1968; Huehns, 1968) though this method does not resolve it from one of the minor components of Hb A. Separation can also be achieved on CM Sephadex (Zade-Oppen, 1963; Honig, 1967).

**Function of Fetal Haemoglobin.** The oxygen affinity curves of human and most mammalian fetal and avian embryonic bloods lie to the left of the corresponding curves for adult blood (Darling et al, 1941; Bartels, Hiller, and Reinhardt, 1966). This is also true for suspensions of cells in buffered saline, ie, the fetal cells have a higher oxygen affinity than adult cells. This would be expected on general grounds since the higher affinity of fetal blood would enable it to take up oxygen from the adult blood in the maternal circulation. Purified human Hb F has been reported to show the same oxygen affinity (Allen, Wyman, and Smith, 1953) or a lower affinity than purified Hb A (McCarthy, 1943; Tiyuma and Shimizu, 1969). The explanation why the human fetal cells nevertheless show a higher oxygen affinity lies on the different responses of Hb A and Hb F to 2,3 DPG. Hb A binds 2,3 DPG strongly and its oxygen affinity is considerably reduced; Hb F binds 2,3 DPG less strongly. In the presence of equivalent amounts of 2,3 DPG the oxygen affinity of Hb A is reduced much more than that of Hb F (Tiyuma and Shimizu, 1969). Fetal and adult cells have approximately the same concentration of 2,3 DPG (Guest and Rapoport, 1941) and consequently fetal cells have a higher oxygen affinity than adult cells. A further factor assisting oxygen transfer across the placenta is the change in pH occurring in the fetal and maternal blood. Fetal blood reaching the placenta undergoes a rise in pH from 7.3 to 7.4 increasing its oxygen affinity (Bohr effect) and assisting oxygen uptake. Maternal blood undergoes a fall in pH assisting its release (Walker, 1959).

**Conditions in which there is a Retention or Reappearance of Hb F in Adult Life**

Normally Hb F is replaced virtually completely by Hb A within about one year after birth. However in a number of abnormal or pathological conditions the synthesis of Hb F may persist into adult life or may reappear.

**Hereditary Persistence of Fetal Haemoglobin (HPFH).** A benign hereditary condition in which the synthesis of Hb F persists into adult life was first described by Edington and Lehmann (1955a and b) in Ghana. It was subsequently found in Uganda, Jamaica, USA, Greece, and Italy (summarized by Ringelhann et al, 1970) and in Thailand (Wasi, Pootarakul, and Na-Nakorn, 1968). The condition occurs with a frequency of about 0.1% in the Negro population in Baltimore and Boston (USA) and of about 0.25% in Greece. Negro heterozygotes have about 25% Hb F whereas the Greek heterozygotes have about 14% Hb F (Fessas and Stamatoyanopoulos, 1964). The haemoglobin is uniformly distributed. The trait is not associated with any other haemological abnormality. Another type of HPFH with only 2-3% Hb F was found in about 1% of about 3000 recruits from southern Switzerland (Marti and Büelter, 1961). Homozygotes for HPFH have been found (Wheeler and Krevans, 1961; Polosa et al, 1964;
Ringelhann et al., 1970); the cases described by Wheeler and Krevans and Polosa were quite young children (three to four years old at the time of investigation) but the person described by Ringelhann and his co-workers was a 45-year-old man.

**Persistence of Fetal Haemoglobin in Haemoglobinopathies.** Persistence of synthesis of small amounts of Hb F has been observed in haemoglobinopathies affecting the synthesis of \( \beta \) chains (Beaven, Ellis, and White, 1960b). In homozygous sickle-cell disease Hb F is found in adults (Singer et al., 1951) in amounts ranging from about 2% up to 20–30% (Beaven, Ellis, and White, 1961). The Hb F is not distributed uniformly but varies from cell to cell (Bertles and Milner, 1968). Hb F levels are reported to decrease with age in men but not in women (Bickers, 1966). G. R. Serjeant (personal communication) also observed that female SS homozygotes tend to have somewhat higher levels of Hb F than male. This, and other findings discussed below, suggest that Hb F synthesis may be subject to some degree of hormonal control.

In heterozygous \( \beta \) thalassaemia, which causes a variable degree of suppression of \( \beta \)-chain synthesis, there is a moderately elevated level of Hb F not usually exceeding about 6% which is unevenly distributed through the erythrocyte population. In heterozygous \( \beta \delta \) thalassaemia (F thalassaemia) which causes suppression of both \( \beta \)- and \( \delta \)-chain synthesis there is a more marked increase of Hb F up to about 35% (reviewed by Motulsky, 1964). In homozygous thalassaemia where there is more severe or complete suppression of \( \beta \)-chain synthesis Hb F may constitute 80–90% of the total haemoglobin.

**Hb F in Chromosomal Abnormalities.** There is a delayed postnatal disappearance of Hb F in infants with \( D_1 \) (13) trisomy and the appearance of Hb A\(_2\) is slightly delayed (Huehns et al., 1964d; Powars, Rohde, and Graves, 1964). Trace amounts of Hb Gower 2 were also found. These findings have suggested that some structural or control genes affecting haemoglobin synthesis might be located on the chromosome triplicated in \( D_1 \) (13) trisomy (Huehns et al., 1964d).

**Reappearance of Hb F.** Reappearance of Hb F (Beaven, Ellis, and White, 1960a) has been observed in acquired aplastic anaemia, leukaemia, pernicious anaemia, and in certain cases of excessive endocrine activity eg, thyrotoxicosis.

High levels of Hb F, up to 50%, have been observed in acute granulocytic leukaemia of children below the age of five years (Beaven and White, 1963; Hardisty, Speed, and Till, 1964). Two forms of childhood myelocytic leukaemia are recognized (Hardisty et al., 1964). A juvenile type is associated with a high level of Hb F, absence of the Philadelphia chromosome and a poor response to therapy. An adult type is associated with the presence of the Philadelphia chromosome, low levels of Hb F, and a better response to therapy. Weatherall, Edwards, and Donohoe (1968) observed a gradual reversion of haemoglobin and carbonic anhydrase to the fetal type in a study of one patient, a 2-year-old girl, with juvenile myelocytic leukaemia. The I antigen titre (see below) also fell to values approaching that characteristic of cord blood.

Slightly increased amounts of Hb F (1–3%) have been found in children with lymphoblastic leukaemia and monocytic leukaemia (Weatherall et al., 1968).

Small amounts (1–2%) of Hb F were reported in approximately 50% of patients with untreated Addisonian pernicious anaemia (Beaven et al., 1960b). In some cases there was an initial rise in Hb F level followed by a fall to normal levels in response to vitamin B12 therapy.

**Maternal Synthesis of Hb F in Pregnancy.** Pembrey and Weatherall (1971) in a short report observed that of 45 women followed throughout pregnancy and six months after, 20–25% had a raised level of Hb F in the second trimester, which fell to normal at 36 weeks. The rate of increase and non-uniform distribution suggested that the Hb F was of maternal origin. This was confirmed by *in-vitro* labelling studies of reticulocytes of women in the first trimester of pregnancy. The results suggested that a factor might be synthesized in early pregnancy which is capable of inducing fetal haemoglobin synthesis.

Rucknagel and Chernoff (1955) also reported slightly raised levels (2.8–3.5%) of Hb F in about 10% of women during the second trimester of pregnancy. Bromberg, Salzburger, and Abrahamov (1957) reported that Hb F was raised in four patients with molar pregnancy. These patients excreted large amounts of chorionic gonadotrophin. Since no fetus was present in these cases, the source of the Hb F must have been the mother.

In an attempt to investigate possible hormonal influences on fetal haemoglobin, Davies and Bull (1971) reported that the administration of prolactin to hamsters induced the production of a haem protein with the same electrophoretic mobility as hamster fetal haemoglobin. The authors tested a number of tranquilizing drugs which have been reported to induce lactation or breast enlargement
and which might presumably induce endogenous prolactin production. Most of the drugs were inactive in inducing the production of the haem proteins, and two, fluphenazine and imipramine, induced very small amounts.

Lie Injo, Hollander, and Fudenberg (1967) found three patients with increased Hb F (2-9, 5-1, and 19-5%) in 13 patients with thyrotoxicosis. Nine patients had a deficiency of carbonic anhydrase activity, another characteristic of fetal erythrocytes. The patient with the high level (19-5%) of Hb F was treated for thyrotoxicosis and showed a decrease of Hb F to 4-4% and a rise in carbonic anhydrase activity to normal levels.

In all cases in which reappearance of Hb F is observed, there is a heterogenous distribution of Hb F among the erythrocyte population, this also applies in thalassaemia and in haemoglobinopathies (Shepard, Weatherall, and Conley, 1962). However in hereditary persistence of fetal haemoglobin the Hb F is always uniformly distributed.

Other Changes Occurring During the Switch from Fetal to Adult Haemoglobin. The change from the production of fetal to adult haemoglobin is accompanied by changes in some of the enzymes present in the cell and in other properties of the cell. Cord blood cells have a MCV about 20% larger than that of cells from adults (Neerhout, 1968) and contain more K + and water (Zipursky, Larue, and Israels, 1960). Cells from newborns have a higher lipid content although the proportions of the lipid components are similar to that in adult cells with only minor variations in the proportions of various phospholipid fractions and of fatty acids (Neerhout, 1968). There is a lower level of carbonic anhydrase and a different hexokinase isoenzyme in cord blood cells (Holmes et al, 1967).

Hexokinase activity in human erythrocytes can be separated into three distinct bands by starch gel electrophoresis at pH 8.6; the bands are designated I, II, and III in order of increasing electrophoretic mobility. Normal adult erythrocytes contain hexokinase types I and III. Type II is present in the erythrocytes of newborn infants and is absent from those of normal adults. It is, however, present in the erythrocytes of adults with hereditary persistence of Hb F. Type II hexokinase and Hb F appeared to be associated. Neonatal erythrocytes are also reported to be more susceptible to haemolysis in dilute H 2 O 2 than adult erythrocytes (Gordon, Nitowsky, and Cornblath, 1955), this is presumably due to the lower levels of catalase and glutathione peroxidase (Gross et al, 1967). Jones and McCance (1949) found significantly reduced levels of true cholinesterase, glyoxylase, carbonic anhydrase, and catalase from cord blood cells compared to the levels in cells from adults. Adult blood cells have an antigen (I antigen) which is absent from cord cells (McGinniss, Schmidt, and Carbone, 1964). Cord blood cells have a different antigen, the i antigen, which is maximally developed at birth and decreases during the first year of life (Marsh, 1961). The increase of i antigen is associated with a reciprocal increase in I antigen which is poorly developed at birth. A high i antigen titre and the fetal type II hexokinase isoenzyme have been found in an individual homozygous for HPFH (Charache, Schruefer, and Bias, 1968). Increased anti-i activity without any corresponding decrease in I antigen has been reported in patients with β thalassaemia major (Giblett and Crookston, 1964). Increased anti-i activity has also been reported in patients with hypoplastic anaemia and acute leukaemia. There was no correlation between the amount of Hb F and i antigen.

Changes in the Ratio of γ and δγ Chains. One of the most interesting recent developments has been the discovery of multiple structural genes for the γ chain of fetal haemoglobin (Schroeder et al, 1968). When globin from Hb F is treated with cyanogen (CNBr) the methionyl bands are cleared. One of the resulting peptide fragments γCB3 contains residues 134-146 of the γ chain with the amino-acid sequence shown in Figure 1. Amino-acid analysis of this peptide gave integral values for all the amino acids except glycine and alanine. Analysis of γCB3 from many cord bloods gave average values close to Gly0.75 and Ala2.25. This indicated that there might be two types of γ chain; one with glycine and one with alanine at position 136. The γCB3 obtained from normal cord blood evidently contained a mixture of the two types. If the composition of γCB3 were Glyγ Alaγ then the chains would all be of the δγ type; if it were Gly, Ala2 they would all be of δγ type. Intermediate values of glycine indicate a mixture of the two types. Evidence that the two types of chain were the products of distinct genes rather than, for example, the result of ambiguous translation, is provided by analysis of mutants of Hb F where the abnormal γ chain must be the product of a single gene. In the cases where this has been examined it has been found that there is either all alanine or all glycine at position 136 and not a mixture. The ratio of δγ to δγ has been studied in Hb F from cases of HPFH in Negroes (Huismans et al, 1969b) and Greeks (Huismans et al, 1970); in the Hb F from β thalassaemia (Schroeder et al, 1970; Schroeder and
Huisman, 1970) and combinations of HPFH and β thalassaemia (Huisman et al, 1971), from F thalasaemia (Stamatoyannopoulos et al, 1971), in acquired haematological disorders (Rosa, Beuzard, and Toulogaot, 1971) and the postnatal changes in the \( \alpha^0/\gamma \) ratio have been studied by Schroeder et al (1971). The results of some of this work have been summarized by Huisman and Schroeder (1971). Analysis of 94 cord blood samples from various parts of the world give mean values of 0.72 and 2.32 for the numbers of glycine and alanine residues in \( \gamma \)-CB3. The phenomenon appears to be universal for the samples were taken from babies of a wide variety of races. The ratio \( \alpha^\gamma \) to \( \gamma \) changes during the period after birth and eventually attains a ratio of approximately 2:3. This ratio is also characteristic of Hb F isolated from normal adults. At present it is difficult to account for how this change in ratio might come about. Huisman and Schroeder (1971) speculate that perhaps similar mechanisms control not only the ratio of the \( \alpha^\gamma \) and \( \gamma \) chains but also that of the \( \beta \) and \( \delta \) chains.

Some interesting differences have been observed in \( \alpha^\gamma/\gamma \) ratio in Hb F from heterozygotes and homozygotes for HPFH and double heterozygotes for HPFH and β thalassaemia. Negro heterozygotes for HPFH produced Hb F containing only the \( \alpha^\gamma \) chain. There were two subgroups, one producing on average about 5% Hb F and another producing 10–20% Hb F (average 13.5%); one person who was a double heterozygote for Hb S and HPFH produced 30% Hb F containing only the \( \alpha^\gamma \) chain. On the other hand, Greek HPFH heterozygotes produced Hb F containing only \( \gamma \) chains; again there were two subclasses—one producing on average about 5% Hb F the other about 17% Hb F. The majority of HPFH heterozygotes produced Hb F containing both types of chains. These were subdivided into three groups. In the major group there was 20–30% Hb F containing an average of 0.4 residues of glycine; a second, smaller group of individuals producing the same amount of Hb F but with a higher average value of 0.6 residues of glycine. A third subgroup produced less Hb F, about 15%, containing only about 0.15 residues of glycine. It was observed that when a \( \beta^\alpha \) gene was in trans to the HPFH determinant in the major group the amount of Hb F produced was increased but the \( \alpha^\gamma/\gamma \) ratio was unchanged. In a family in

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**Fig. 1.** Amino-acid sequence of the \( \gamma \) chain of human Hb F*

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>10</th>
<th>20</th>
<th>30</th>
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<th>50</th>
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<td>PRO</td>
<td>TRP</td>
<td>THR</td>
<td>ALA</td>
</tr>
</tbody>
</table>

* The residues which differ in the \( \beta \) and \( \gamma \) chains are given in italics. The corresponding residue in the \( \beta \) chain is shown beneath. Position 136 is occupied by either glycine or alanine.

**Amino-acid differences between the \( \beta \) and \( \gamma \) chains.** (The remaining differences are at external sites.)

1. Internally sited residues
2. \( \alpha_{1}\beta_{1} \) contact
3. Central cavity
4. DPG-binding site

<table>
<thead>
<tr>
<th>Positions</th>
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<th>Alanine</th>
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<td>1, 143</td>
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</tbody>
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\( \gamma \)-CB3 -
which there was a combination of HPFH and \( \beta \) thalassaemia the \( \alpha_\gamma/\alpha_\gamma \) ratio in double heterozygotes for HPFH and \( \beta \) thalassaemia was intermediate between the \( \alpha_\gamma/\alpha_\gamma \) ratio in single heterozygotes for HPFH and for \( \beta \) thalassaemia. In the various acquired haematological disorders exhibiting raised Hb F studied by Rosa et al (1971) the amount of glycine in \( \gamma \)CB3 varied between about 0.5 and 0.94 residues. There was no obvious correlation between the nature of the disease, the amount of Hb F synthesized, the state of the bone marrow, or the nature of the treatment. Two embryos of 16 and 20 weeks were also examined, they showed the same \( \alpha_\gamma/\alpha_\gamma \) ratio as newborns.

The observations of Hb F in the HPFH condition have been explained by assuming deletions of various gene loci (Huisman et al, 1969b). However, these schemes do not explain some important observations particularly the change in \( \alpha_\gamma/\alpha_\gamma \) ratio after birth and the heterogeneity of the condition.

Clearly the situation is complex, but it now seems well established that there are at least two genetic loci for the \( \gamma \) chain; possibly there may be as many as four (Huisman et al, 1972). The different loci are capable of being expressed to different extents during development and in some abnormal conditions. These important observations still await a full explanation which will undoubtedly greatly increase our understanding of the factors regulating haemoglobin synthesis.

There is some evidence indicating that the \( \alpha \)-chain locus is duplicated (Lehmann and Carrell, 1968). It is frequently observed that abnormal human haemoglobins with mutations in the \( \alpha \) chain make up about a quarter of the total haemoglobin whereas \( \beta \)-chain mutants make up nearer to half the total in heterozygotes. The \( \beta \) chain is known to be the product of a single gene locus: if there were two \( \alpha \)-chain gene loci then the abnormal \( \alpha \) chain would be expected to amount to about a quarter of the total if all genes were expressed equally. The most convincing evidence for there being at least two \( \alpha \)-chain loci comes from the observation by Hollan et al (1972) of an individual whose blood contained two different \( \alpha \)-chain abnormal haemoglobins in addition to normal Hb A. As three different \( \alpha \) chains cannot arise from a single locus, there must be at least two \( \alpha \)-chain loci in this individual. However, individuals have been found in New Guinea (Beaven et al, 1972) who appear to be homozygous for the \( \alpha \)-chain abnormal haemoglobin J\( a \) Tongariki. They did not appear to be suffering from \( \alpha \) thalassaemia and since it is highly improbable that they could have inherited four abnormal \( \alpha \)-chain genes they may have only a single \( \alpha \)-chain gene locus. It is possible that there might be a heterogeneity within the population, some individuals having a single \( \alpha \)-chain locus and others two loci.

**Haemoglobin Changes Occurring in Sheep and Goats in Response to Anaemia**

A somewhat different change in haemoglobin type occurs in sheep and goats in response to anaemia. Sheep may be classified into three main genotypes with respect to their adult haemoglobins; AA producing only Hb A, AB producing Hb A and Hb B, and BB producing only Hb B. Haemoglobins A and B have a common \( \alpha \) chain and differ in their \( \beta \) chains, Hb B has a higher oxygen affinity than Hb A. An additional rare allele of the \( \alpha \) chain (\( \alpha^0 \)) occurs in some sheep; the \( \alpha^0 \) chain differs from the normal \( \alpha \) chain by a Gly\( \rightarrow \)Asp substitution at position 15 (Huisman et al, 1968). When sheep of genotypes AA and AB are made anaemic by repeated bleeding a new type of haemoglobin, Hb C, appears. This change can also be induced in non-anaemic sheep by injection of plasma from anaemic sheep (Boyer, 1967; Gabuzda et al, 1968). Sheep Hb C contains a new type of \( \beta \) chain the \( \beta^c \) chain which contains only 141 amino-acid residues and differs from the \( \beta^a \) chain in at least 16 positions. Animals containing only the \( \beta^b \) gene do not produce Hb C. Hb C has also been found in young lambs in the first few months after birth (Huisman et al, 1969a). At birth the haemoglobin was 95–98% Hb F, this decreased after birth and none was detectable at 40 days. At birth no Hb C was detectable, but the level rose during the 2 weeks following birth and reached 5–6% at 14–20 days and afterwards fell, reaching about 1.2% at 76 days. Other haematological changes were observed during this period. There was an initial fall in PCV and haemoglobin concentration and an increase in the number of reticulocytes during the first 20 days. The production of Hb C was stimulated by injected erythropoietin.

The same phenomenon was observed to a much more marked degree in young goats. Adult goats of genotypes AA, AB, and BB all have a common \( \beta \) chain and differ in their \( \alpha \) chains. A variant of \( \beta \) chain (\( \beta^b \)) is found in some goats, the \( \beta^b \) chain differs from the normal \( \beta \) chain by an Asp\( \rightarrow \)His substitution at position 21. When goats are made severely anaemic, a new type of chain, the \( \beta^c \) chain is produced in all phenotypes including those with the \( \beta^b \) chains. The \( \beta^c \) of the goat is very similar to that of the sheep and possibly differs only by a Thr\( \rightarrow \)Ser substitution at position 50 (Huisman et al, 1967). The \( \beta^c \) chain is present in small amounts in non-anaemic goats. In newborn goats Hb F constituted
86–98% of the haemoglobin in 12 animals examined by Huisman et al (1969a). Haemoglobins containing $\beta^c$ chains were virtually absent at birth but rapidly increased in amount after birth and by 40–50 days constituted more than 80% of the total haemoglobin. Haemoglobins containing $\beta^c$ chains showed a slight rise in the first 4–10 days followed by a decline to very low levels. After 60 days the $\beta^c$-containing haemoglobins were gradually replaced by $\beta^A$-containing haemoglobins. During this initial period of growth of the animals other haematological changes occurred. There was a large increase in red blood cell count from about $9 \times 10^6$ per mm$^3$ to more than $20 \times 10^6$ per mm$^3$, a decrease in MCV and a rise in the number of reticulocytes. Injection of extracts containing erythroid-stimulating factors stimulated the production of the $\beta^c$ chain and led to almost complete formation of the $\beta^c$ chain. In sheep similar experiments produced only about 10% of haemoglobin containing the $\beta^c$ chain.

These findings are of great interest in that they indicate that the production of different haemoglobin types may be subject to hormonal control.

Conclusion

Further studies of the developmental changes in haemoglobin and of the accompanying changes in the red cell should continue to be a fruitful field of research. The means by which these changes are brought about and the causes of the reversion to fetal haemoglobin production in certain disorders are not understood. Some evidence suggests that the change may be subject to hormonal control and the evidence is stronger in the case of the related changes occurring in sheep and goats. A fuller understanding of these processes might provide a basis for the treatment of some serious haematological disorders such as sickle-cell and $\beta$ thalassaemia.

References


Is there an 'embryonic' or 'primitive' hemoglobin? Blood, 16, 948-996.


