Human Haemoglobins

E. R. HUEHNS* and E. M. SHOOTER

From Medical Research Council Group in Haemolytic Anaemia, University College Hospital Medical School and Department of Biochemistry, University College London, and Department of Genetics, Stanford University Medical Center, Palo Alto, California, U.S.A.

I—Structure of Haemoglobin II—The Genetic Control of Haemoglobin Synthesis III—The Biosynthesis of Haemoglobin IV—Clinical Features of Abnormalities in Haemoglobin Synthesis

During the past 15 years the study of the normal and abnormal human haemoglobins has advanced our understanding of the genetic control of protein synthesis and also provided an example of the various ways in which abnormalities of protein synthesis can cause disease. There have been a number of reviews on this subject (Itano, Bergren, and Sturgeon, 1956; Itano, 1957; Beaven and Gratzer, 1959; Lehmann and Ager, 1960; Bannerman, 1961; Hill, 1961; Ingram, 1961; Rucknagel and Neel, 1961; Baglioni, 1963a; Jonxis, 1963), and this review mentions only the more important early advances, but deals in more detail with those of present interest.

I: STRUCTURE OF HAEMOGLOBIN

Haemoglobins are large, complex, protein molecules, the function of which is to transport oxygen from the lungs to the tissues. Recent work by Perutz and his collaborators (Perutz, Rossman, Cullis, Muirhead, Will, and North, 1960; Cullis, Muirhead, Perutz, Rossman, and North, 1962) has revealed the over-all arrangement of the molecules, and the model these workers have built on the basis of their x-ray crystallographic work is shown in Fig. 1 and 2. In over-all shape, the molecule resembles a spheroid $64 \text{ \AA} \times 55 \text{ \AA} \times 50 \text{ \AA}$. It can be seen that each molecule consists of four polypeptide chains and that a haem group is

attached to each chain. Each molecule consists of two pairs of identical chains, called α - and β -chains, so that its structure can be written $\alpha_2\beta_2$. In Fig. 1 and 2, the two α -chains are in white, while the two β -chains are in black. The internal arrangement of the two types of chain is very similar, the main difference being that the β -chain is slightly longer, and this is confirmed by the amino acid sequence studies described later. The model further shows that there is relatively little contact between pairs of like chains, whereas there is striking complementarity between adjacent surfaces of α - and β -chains. The haem groups are not particularly close to one another, and their arrangement offers no clue to the influence they exert on each other in the oxygenation reaction. Recently, however, Muirhead and Perutz (1963) and Perutz (1964) have found that the arrangement of the subunits alters during oxygenation. It appears that the β -chains move closer together on oxygenation, decreasing the distance between their haem groups by 7 $\text{\AA}$.

The haem groups lie in four separate pockets on the surface of the molecule, each pocket being formed by the folds of one of the four polypeptide chains. Each haem group is attached to its polypeptide chain by a co-ordinate linkage from the iron of the haem group to a specific histidine residue at position 87 in the amino acid sequence in the α -chain and at position 92 in the β -chain. Another histidine occurs in each polypeptide chain on the opposite side of the haem group (58 α and 63 β) and is linked to the iron of the haem

Received November 13, 1964.

* Senior Beit Memorial Fellow.

group through either an oxygen molecule in oxyhaemoglobin or a water molecule in reduced haemoglobin.

Normal Human Haemoglobins

There are several normal human haemoglobins at different stages of human development: adult, foetal, and embryonic.

Haemoglobins Found in Adult Life

HAEMOGLOBIN A. As explained above, Hb-A consists of two α^A -chains joined to two β^A -chains. The formula for Hb-A can thus be written $\alpha_2^A\beta_2^A$ (superscripts, as, for example, α^A , designate the source of each chain, while subscripts denote the number of each type of chain present (Schroeder, 1959)). The amino acid sequences of each chain are given in Fig. 3. When the sequences of the two chains are compared, it is found that the β -chain has 5 amino acids more than the α -chain and, although many parts of the amino acid sequence are the same, there are approximately 84 differences between the two chains. The molecular weight of the haemoglobin molecule calculated from the above structure is 64,500.

HAEMOGLOBIN A₂. Hb-A₂ occurs in all normal haemolysates after the postnatal period and amounts to 1.5–3% of total haemoglobin (Kunkel, Ceppellini, Müller-Eberhard, and Wolf, 1957). It is distinguished from Hb-A by its slower electrophoretic migration towards the anode and can be separated by all the conventional systems. It can also be separated by chromatography on carboxymethylcellulose (CMC) (Huisman, Martis, and Dozy, 1958), diethylaminoethylcellulose (DEAE) (Huisman and Dozy, 1962) and Amberlite IRC 50 (Schnek and Schroeder, 1961). A convenient two-step preparative method has also been described (Huehns and Shooter, 1961b). In its alkali denaturation rate and absorption spectrum, Hb-A₂ resembles Hb-A.

FIG. 1. The structure of horse haemoglobin at 5.5 Å showing the arrangement of the four subunits. The white chains are α -chains and the black β -chains (from Perutz *et al.* (1960) with permission).

FIG. 2. A similar model to that in Fig. 1 with one of the white α -chains omitted to show the close contact between white α - and black β -chain subunits (from Perutz *et al.* (1960) with permission).

FIG. 3. The amino acid sequence of the α^A - and β^A -chains (compiled by Schroeder (1963) from the data of Braunitzer, Gehring-Müller, Hilschmann, Hilse, Hobom, Rudloff, and Wittman-Liebold (1961); Konigsberg and Hill (1962); Schroeder, Shelton, Shelton, and Cornick (1962)). The amino acids that are identical in the two chains are indicated by capital letters.

TABLE I

AMINO ACID DIFFERENCES OF δ -CHAIN OF Hb-A₂ COMPARED WITH β^A -CHAINS (Ingram and Stretton, 1962a, b; Hill and Kraus 1963; Jones, 1964)

Position in Amino Acid Sequence	Residue in β^A -chain	Residue in δ^A -chain
9	Ser	Thr
12	Thr	AspNH ₂
22	Glu	Ala
50	Thr	Ser
86?	Ala	Ser
87?	Thr	GluNH ₂
116	His	Arg
117	His	AspNH ₂
125 (or 124)	Pro	GluNH ₂
126	Val	Met

Hb-A₂, like other haemoglobins, consists of four polypeptide chains, two of which are identical to the α^A -chains (Muller and Jonxis, 1960; Huehns and Shooter, 1961a, b; Ingram and Stretton, 1961; 1962a, b). The other two are very similar to the β -chains but differ by ten amino acid substitutions (Table I) (Ingram and Stretton, 1961; 1962a, b; Hill and Kraus, 1963; Jones, 1964). These non- α -chains have been called δ -chains, and the structure of Hb-A₂ is therefore $\alpha_2^A\delta_2^A$.

OTHER MINOR ADULT HAEMOGLOBINS. Several other minor haemoglobin components are found in adult haemolysates. Hb-A₃, which forms approximately 10% of total haemoglobin (Kunkel and Wallenius, 1955), appears to be derived from Hb-A during *in vivo* ageing of the red cells (Kunkel and Bearn, 1957; Meyering, Israels, Sebens, and Huisman, 1960). Hybridization of Hb-A₃ shows that it differs from Hb-A in the β -chains (Rosa and Labie, 1962). More recent work indicates

that Hb-A₃ is a mixture of haemoglobins (Schnek and Schroeder, 1961; Huisman and Meyering, 1960). Schnek and Schroeder (1961) have detected several minor components in normal haemolysates by chromatography on IRC 50 and have called these Hb-A_{1a}, 1b, 1c, 1d, 1e. The largest of these, Hb-A_{1c}, amounting to approximately 5% of total haemoglobin, has the structure $\alpha_2^A\beta^A\beta^X$, the β^X -chain differing from the β^A -chain by the substitution of the terminal NH₂ group (Holmquist and Schroeder, 1964). It has also been suggested that part of the Hb-A₃ fraction is composed of a complex of oxidized glutathione (GSSG) with Hb-A (Muller, 1961; Huisman and Dozy, 1962). However, it is not known whether this complex is present *in vivo*. Hybridization experiments show that the GSSG is attached to the β -chains.

A small quantity of foetal haemoglobin is also present in adult red cells (Chernoff, 1953b), but this does not amount to more than 0.4% (Beaven, Ellis, and White, 1960b).

Haemoglobins in Foetal Life.

FOETAL HAEMOGLOBIN. Hb-F is the major pigment found in the red cells during foetal life and differs in several respects from Hb-A. It was first detected in 1866 by Körber, who noticed that this pigment was denatured more slowly by alkali than that found in adult red cells.

Foetal haemoglobin, like Hb-A, consists of four polypeptide chains. Two of these are identical to the α^A -chains (Schroeder and Matsuda, 1958; Hunt, 1959; Schroeder, 1963), while the other two chains have the same number of amino acids as the β -chains but differ from them in 39 amino acid residues and are called γ -chains. The structure of Hb-F is thus $\alpha_2^A\gamma_2^F$. The amino acid sequence of the γ -chains is given in Fig. 4.

Hb-F differs from Hb-A by its greater resistance to alkali denaturation (Körber, 1866), and this property is used to measure the amount of Hb-F in various haemolysates (Singer, Chernoff, and Singer, 1951; Beaven, Ellis and White, 1960a; Betke, Marti, and Schlicht, 1959). The haem absorption spectrum of Hb-F is identical to that of Hb-A. However, there is a distinct difference in the protein absorption at about 290 m μ . In Hb-F the tryptophan fine structure band is resolved at 2,896 Å, while in Hb-A it is unresolved at 2,910 Å (Jope, 1949; Beaven *et al.*, 1960a), the difference arising from the altered tryptophan/tyrosine ratios of the two haemoglobins.

Hb-F can be separated from Hb-A by electrophoresis either in agar gel, using a sodium citrate

GLY-his-PHE-thr-GLU-glu-ASP-lys-ALA-THR-ILEU- thr-SER-
 leu-try-gly-lys-val-asn-val-GLU-ASP-ALA-gly-gly-glu-
 THR-leu-gly-arg-leu-leu-val-val-tyr-pro-try-thr-gln-arg-
 phe-phe-ASP-ser-phe-gly-ASN-leu-ser-SER-ALA-SER-ala-
 ILEU-met-gly-asn-pro-lys-val-lys-ala-his-gly-lys-lys-val-
 leu-THR-SER-LEU-GLY-asp-ALA-ILEU-LYS-his-leu-asp-ASP-
 leu-lys-gly-thr-phe-ala-GLN-leu-ser-glu-leu-his-cys-asp-
 lys-leu-his-val-asp-pro-glu-asn-phe-LYS-leu-leu-gly-asn-
 val-leu-val-THR-val-leu-ala-ILEU-his-phe-gly-lys-glu-
 phe-thr-pro-GLU-val-gln-ala-SER-TRY-gln-lys-MET-val-
 THR-gly-val-ala-SER-ala-leu-SER-SER-ARG-tyr-his

FIG. 4. The amino acid sequence of the γ^F chain. The residues that differ from those in the β^A chain have been capitalized (Schroeder, 1963).

buffer at pH 6.2 (Robinson, Robson, Harrison, and Zuelzer, 1957), or in starch gel with a tris-E.D.T.A.-borate buffer at pH 8.6 (O. Smithies, unpublished). In other electrophoretic systems, consistent separation of Hb-A and Hb-F is difficult to achieve. Chromatographically, Hb-F is most conveniently separated on IRC 50 resin, using a sodium phosphate buffer at approximately pH 7.0 (Allen, Schroeder, and Balog, 1958). This method is useful for the preparation of pure Hb-F.

MINOR FOETAL HAEMOGLOBINS. Several minor haemoglobins are present in foetal blood. The largest of these is Hb-F₁ (Allen *et al.*, 1958), which amounts to approximately 10% of total haemoglobin. This has the structure $\alpha_2^A\gamma^F\gamma^X$, the γ^X -chain differing from the γ^F -chain by the substitution of the terminal NH₂ group with an acetyl group (Schroeder, Cua, Matsuda, and Fenninger, 1962). A small amount of Hb- γ_4 is also detected by starch gel electrophoresis (Karaklis and Fessas, 1963) and amounts to approximately 0.3% of total haemoglobin in normal cord bloods (Huehns, Dance, Beaven, Hecht, and Motulsky, 1964b).

After birth, the proportion of Hb-F found in haemolysates rapidly falls (Fig. 5), only traces being present after the first year of life (White and Beaven, 1959).

Embryonic Haemoglobins. The possibility of the existence of a human embryonic haemoglobin was first suggested by Drescher and Künzer (1954). Recently, it has been shown by starch gel

FIG. 5. The developmental changes in human haemoglobins.

electrophoresis (Fig. 6) that there are two embryonic haemoglobins, Hb-Gower 1 and Hb-Gower 2 (Huehns, Flynn, Butler, and Beaven, 1961a; Huehns, Dance, Beaven, Keil, Hecht, and Motulsky, 1964a).

Hb-Gower 2 consists of four polypeptide chains. Again, two α^A -chains are present, while the other two chains differ from the β -, γ -, and δ -chains by more than one amino acid substitution and have been called ϵ -chains (Huehns *et al.*, 1964a; Huehns, Hecht, Keil, and Motulsky, 1964c).

Hb-Gower 2 has an alkali denaturation rate intermediate to Hb-A and Hb-F, and the ultraviolet absorption spectrum resembles that of Hb-F rather than that of Hb-A. It has not yet been possible to study Hb-Gower 1 in detail, but it has been suggested that it consists solely of ϵ -chains.

In the youngest embryo studied, which had a crown-rump (C.R.) measurement of 25 mm., the haemolysate contained mainly Hb-F, 24% Hb-Gower 1, 13% Hb-Gower 2, approximately 5% Hb-A, and 1% Hb- γ_4 . The amount of these

FIG. 6. Starch gel electrophoresis in a *tris*-E.D.T.A.-borate buffer pH 8.6: (i) haemolysate from 3.5 cm. (crown rump) human embryo; (ii) Hb-A + S marker; (iii) normal cord blood.

embryonic haemoglobins falls rapidly as development proceeds and they are not normally found after the 100 mm. C.R. stage of development (Fig. 5).

The Abnormal Haemoglobins

The discovery by Pauling, Itano, Singer, and Wells (1949) that the haemoglobin in sickle cell disease differed from normal haemoglobin in its rate of migration on electrophoresis showed for the first time that an abnormal protein molecule could be the primary cause of a severe disease. Since then, a large number of haemoglobins have been discovered. Some of these have been found through the study of patients with various haematological disorders, but the majority have been discovered during surveys carried out in various parts of the world. These have been distinguished from each other by differences in behaviour on electrophoresis. Some of the abnormal haemo-

globins with similar electrophoretic mobilities have been further differentiated by other methods, for example, by chromatography on IRC 50 (Huisman and Prins, 1955). The properties of the various abnormal haemoglobins have been reviewed by Beaven and Gratzer (1959), Lehmann (1960), and Lehmann and Ager (1960). The relative electrophoretic mobilities of various abnormal haemoglobins are shown in Figs. 7 and 8. More recently, the structure of a number of abnormal haemoglobins has been determined (as outlined in the next section) and on this basis the existence of definitive species has been firmly established (Table II).

The next discovery in the study of the abnormal haemoglobins was made by Ingram (1956, 1958, 1959) and Hunt and Ingram (1958), who showed that in sickle cell haemoglobin, or Hb-S, only the β -chains were abnormal, while the α -chains were identical to those found in Hb-A. The structure of Hb-S could then be written as $\alpha_2\beta^s_2$. Ingram also showed that in each β^s -chain only one amino

FIG. 7. Starch gel electrophoresis in a *tris*-E.D.T.A.-borate buffer pH 8.6: (i) normal adult haemolysate; (ii) Hb-S + C; (iii) Hb-A + S; (iv) normal cord blood; (v) normal adult; and (vi) Hb-A + J. The slight difference in electrophoretic migration between Hb-S in samples (ii) and (iii) is due to the effect of the other haemoglobins present in each haemolysate, illustrating one of the difficulties encountered in interpreting slight differences in electrophoretic mobility (from Huehns and Shooter, 1964a; with kind permission of Blackwell Scientific Publications Ltd.).

FIG. 8. Relative electrophoretic mobilities on starch gel electrophoresis at pH 8.6 of human haemoglobin variants.

acid out of 146 was different, the glutamyl residue which is found in the β^A -chain at position 6 from the N-terminal end being replaced by a valyl residue. A shorthand for this structure gives the number of the amino acid residue substituted and the new amino acid as a superscript to the affected

chains: Hb-S would then be $\alpha_2\beta_2^{A6Val}$ (Gerald and Ingram, 1961).

A similar situation has now been found for almost all other abnormal haemoglobins so far analysed (except those mentioned later), *one amino acid residue being substituted in either the β - or the*

α -chains. Thus, the abnormal haemoglobins fall into two main groups:

- (i) those with abnormal α -chains
- (ii) those with abnormal β -chains,

and these are listed in Table II.

It can be seen from Table II that several types previously thought to be identical are now known to differ; for example, there are at least three structurally different haemoglobins called Hb-D (Benzer, Ingram, and Lehmann, 1958). On the other hand, 5 independently described species of Hb-G $_{\alpha}$ or Hb-D $_{\alpha}$ have been shown to be chemically identical to Hb-G $_{\text{Philadelphia}}$. Three types of Hb-J are known: Hb-J $_{\text{Baltimore}}$, $\alpha_2^A\beta_2^{16\text{Asp}}$ (Baglioni and Weatherall, 1963), Hb-J $_{\text{Rambam}}$, $\alpha_2^A\beta_2^{\text{TpIX}}$ (Salomon, Tatarski, Dance, Huehns, and Shooter, 1964), and Hb-J $_{\text{Oxford}}$, $\alpha_2^J\beta_2^A$ (Beale and Lehmann, 1964). However, the species Hb-J $_{\text{Trinidad}}$ and Hb-J $_{\text{Ireland}}$ (Gammack, Huehns, Lehmann, and Shooter, 1961) are now known to be identical to Hb-J $_{\text{Baltimore}}$ (N. Dance, E. R. Huehns, and E. M. Shooter, unpublished).

Fig. 9 illustrates the distribution of the known amino acid substitutions along the α - and β -chains and indicates that substitutions can occur in most parts of both chains. However, some regions of the chains appear to have more than their fair share of the known anomalies. Several reasons for this can be advanced, but it is perhaps a reflection of the ability of any substitution to cause a change in electrophoretic mobility which would lead to its detection on routine electrophoresis. Thus, substitutions in parts of the polypeptide chain that are buried in the molecule are less likely to be detected than those that are on the surface. In other cases, the presence of disease such as methaemoglobinemia or haemolytic anaemia has led to a number of very detailed studies, revealing the presence of several different abnormal haemoglobins with amino acid substitutions near the haem group.

It will also be seen from Fig. 9 that in some positions more than one substitution has been detected, for example, at position 6 in the β -chains. In Hb-S, valine is found at this site, while in Hb-C, lysine is present. Occasionally, substitutions at corresponding places in the α - and β -chains (allowing for the extra amino acids found in the β -chain) lead to haemoglobins which in their properties are almost indistinguishable. In this class are Hb-J $_{\text{Baltimore}}$ ($\alpha_2^A\beta_2^{16\text{Asp}}$) and Hb-J $_{\text{Oxford}}$ ($\alpha_2^{15\text{Asp}}\beta_2^A$), Hb-O $_{\text{Arabia}}$ ($\alpha_2^A\beta_2^{121\text{Lys}}$) and Hb-O $_{\text{Indonesia}}$ ($\alpha_2^{116\text{Lys}}\beta_2^A$). The significance, if any, of all these findings in relation to the natural occurrence of point mutations in man is not yet clear.

Haemoglobin A₂ Variants. Hb-A₂ has the structure $\alpha_2^A\delta_2^A$, and both α -chain and δ -chain variants have been described.

Hb-B₂, which was the first Hb-A₂ variant discovered (Ceppellini, 1959; Huisman and Meyer-ing, 1960), occurs in approximately 1% of American Negroes. On electrophoresis it migrates more slowly than Hb-A₂ (Fig. 8). Chemical studies indicate that it has abnormal δ -chains (Horton, Payne, Bridges, and Huisman, 1961) and that the amino acid substitution is glycine $\rightarrow$ arginine at position 16 (A. O. W. Stretton, cited by Baglioni, 1963a).

Hb-Flatbush is another Hb-A₂ variant with abnormal δ -chains (Ranney, Jacobs, Bradley, and Cordova, 1963). This haemoglobin migrates on electrophoresis with Hb-S (Fig. 9).

Hb-Sphakia is probably another variant of Hb-A₂ with abnormal δ -chains (C. Krimbas, N. A. Barnicot, and R. B. MacConnell, unpublished). It was found in a single family in Greece. On electrophoresis it migrates between Hb-A₂ and Hb-B₂.

Haemoglobin F Variants. Hb-F $_{\text{Roma}}$ was found in an Italian neonate (Silvestroni and Bianco, 1963). These workers showed that this was a γ -chain variant of Hb-F by hybridization, spectral and alkali denaturation studies. On electrophoresis, Hb-F $_{\text{Roma}}$ migrates more rapidly than either Hb-F or Hb-A towards the anode. Hb-Texas (Schneider, Arat, and Haggard, 1964a) is another γ -chain variant. On electrophoresis, this haemoglobin migrates slightly slower (cathodally) than Hb-C. Chemical studies indicate that a glutamyl residue is replaced by a lysyl residue in tryptic peptide γTpI either at position 5 or position 6 (R. Schneider and R. T. Jones, personal communication). This substitution is the same as that found in Hb-C ($\beta_6\text{Glu} \rightarrow \text{Lys}$) or Hb-C $_{\text{Georgetown}}$ ($\beta_7\text{Glu} \rightarrow \text{Lys}$) and, similarly, occurs near the N-terminal of the polypeptide chain involved. Other γ -chain variants of Hb-F may be Hb-Alexandra (Fessas, Mastrokalos, and Fostiropoulos, 1959; Vella, Ager, and Lehmann, 1959) and Hb-Aegina (Fessas, Karaklis, and Gnafakis, 1961). On electrophoresis Hb-Alexandra migrates with Hb-O, Hb-Aegina slightly faster than Hb-A (Fig. 8).

α -chain variants of Hb-A₂ and Hb-F are found in all subjects who synthesize abnormal α -chains; thus, for example, Hb-G $_{\alpha}$ ($\alpha_2^G\beta_2^A$) is accompanied by its own Hb-A₂ variant, called Hb-G₂ ($\alpha_2^G\delta_2$) (Huehns and Shooter, 1961a) and during foetal life the corresponding α -chain variant of Hb-F,

TABLE II
CHEMICAL DIFFERENCES BETWEEN ABNORMAL HAEMOGLOBINS AND HAEMOGLOBIN A

Abnormal Haemoglobin	Amino Acid Residue Position	Residue in Hb-A	Residue in Abnormal Haemoglobin	Reference
α-CHAIN ABNORMALITIES				
G ^{Philadelphia} = G ^{Bristol} = G ^{Azuokoli} = D ^{St. Louis} = D ^{Washington}	68	Asg	Lys	Baglioni and Ingram, 1961 Dance, Huehns, and Shooter, 1964 Weatherall, Sigler, and Baglioni, 1962
I	16	Lys	Asp	Murayama, 1962a
J ^{Medellin}	22	Gly	Asp	Gottlieb, Restrepo, and Itano, 1964
M ^{Boston}	58	His	Tyr	Gerald and Efron, 1961
M ^{Iwate} = M ^{Kankakee}	87	His	Tyr	Miyaji <i>et al.</i> , 1963a Jones <i>et al.</i> , 1964
O ^{Indonesia}	116	Glu	Lys	Baglioni and Lehmann, 1962
Mexico	54	Glm	Glu	Jones, Koler, and Lisker, 1963
Norfolk	57	Gly	Asp	Baglioni, 1962c
J ^{Oxford}	15	Gly	Asp	Beale and Lehmann, 1964
L ^{Ferrara}	47	Asp	Gly	Baglioni (personal communication)
G ^{Honolulu} = G ^{Singapore} = G ^{Hong Kong}	30	Glu	Gln	Swenson, Hill, Lehmann, and Jim, 1962
Shimonoseki	54	Glm	Arg	Hanada and Rucknagel, 1963; Miyaji, Iuchi, Takeda, and Shibata, 1963b
<i>In the following haemoglobins only the abnormal tryptic peptide has been determined</i>				
D _α	αTpIV			Benzer <i>et al.</i> , 1958
Q	αTpIX			Hill and Jones, cited by Schroeder, 1963
Stanleyville II	'core'			Hall-Craggs <i>et al.</i> , 1964
Beilinson	αTpVI			De Vries, Joshua, Lehmann, Hill, and Fellow, 1963
Kokura (Umi)	αTpVI			Ohya, 1963
<i>In the following haemoglobins only the abnormal polypeptide chains have been determined</i>				
G ^{Ibadan}				Gammack <i>et al.</i> , 1960
K _α				Gammack <i>et al.</i> , 1961
N _α				Silvestroni, Bianco, and Brancati, 1963
Russ				Huisman, 1962; Huisman and Sydenstricker, 1962
Hopkins 2				Itano and Robinson, 1960
β-CHAIN ABNORMALITIES				
S	6	Glu	Val	Ingram, 1959
C	6	Glu	Lys	Hunt and Ingram, 1960
C ^{Georgetown}	7	Glu	Lys	Pierce <i>et al.</i> , 1963
D ^{Punjab} = D ^{Chicago} = D ^{Cyprus} = D ^{Los Angeles}	121	Glu	Glm	Baglioni, 1962a Babin, Jones, and Schroeder, 1964
E	26	Glu	Lys	Hunt and Ingram, 1961
G ^{San Jose}	7	Glu	Gly	Hill, Swenson, and Schwartz, 1960
J ^{Baltimore} = J ^{Ireland} = J ^{Trinidad} = N ^{Newhaven}	16	Gly	Asp	Baglioni and Weatherall, 1963 Gammack <i>et al.</i> , 1960; Dance, Huehns, and Shooter, unpublished Chernoff and Perillie, 1964

TABLE II—continued

Abnormal Haemoglobin	Amino Acid Residue Position	Residue in Hb-A	Residue in Abnormal Haemoglobin	Reference
β-CHAIN ABNORMALITIES				
M ^{Saskatoon} = M ^{emory} = M ^{kurume} = M ^{radom} = M ^{chicago}	63	His	Tyr	Gerald and Efron, 1961 Shibata <i>et al.</i> , 1962a Murawski <i>et al.</i> , 1964; Josephson <i>et al.</i> , 1962; P. Heller, personal communication
M ^{Milwaukee 1}	67	Val	Glu	Gerald and Efron, 1961
O ^{arabia}	121	Glu	Lys	Baglioni and Lehmann, 1962
Zurich	63	His	Arg	Muller and Kingma, 1961
G ^{Galveston} = G ^{Port Arthur} = G ^{Texas}	43	Glu	Ala	Bowman <i>et al.</i> , 1964
Hikari	61	Lys	Asp	Shibata, Miyaji, Iuchi, Ueda, and Takeda, 1964
G ^{accra}	79	Asp	Asp	Lehmann, Beale, and Boi-Doku, 1964
G ^{Coushatta}	22 or 26 (βTpIII)	Glu	Ala	Schneider <i>et al.</i> , 1964b
Seattle	70 or 76 (βTpIX)	Ala	Glu	Huehns, Hartmann, Hecht, and Motulsky, unpublished
Rambam	69 or 74 (βTpIX)	? Gly	? Asp	Salomon <i>et al.</i> , 1964
Kenwood	143	His	Glu or Asp	Bayrakci, Josephson, Singer, Heller, and Coleman, 1964
<i>In these haemoglobins only the abnormal tryptic peptide has been determined</i>				
Köln	'Core'			Hutchinson <i>et al.</i> , 1964
St. Mary's	? 'Core'			Buchanan <i>et al.</i> (unpublished)
R (Durham 1)	βTpI			Chernoff and Liu, 1961
D _β	βTpIII			Benzer <i>et al.</i> , 1958
<i>In these haemoglobins only the abnormal polypeptide chain has been determined</i>				
M ^{Milwaukee 2}				Gerald and Efron, 1961
K _β				O'Gorman, Allsopp, Lehmann, and Sukumaran, 1963
L _β				Gammack <i>et al.</i> , 1961
P				Silvestroni <i>et al.</i> , 1963

Hb-G F ($\alpha_2^G\gamma_2^F$), is found (Minnich, Cordonnier, Williams, and Moore, 1962; see also later for further references).

Haemoglobins with Unusual Structures.

(a) **HAEMOGLOBINS WITHOUT α -CHAINS.** Three haemoglobins without α -chains have been described, corresponding to the normal haemoglobins A, F, and A₂. All these haemoglobins migrate on electrophoresis more rapidly towards the anode than their α -chain-containing parent species, and this difference in electrophoretic mobility is more marked near neutral pH. Hb-H, the first of these to be discovered (Rigas, Koler, and

Osgood, 1955, 1956; Goultas, Fessas, Tseveris, and Xefteri, 1955), consists of four β^A -chains: Hb- β_4^A (Jones, Schroeder, Balog, and Vinograd, 1959). Fast foetal haemoglobin (Fessas and Papaspyrou, 1957) or Hb-Bart's (Ager and Lehmann, 1958) consists of four γ^F -chains, Hb- γ_4^F (Hunt and Lehmann, 1959; Kekwick and Lehmann, 1960), and Hb- δ consists solely of δ -chains (Dance and Huehns, 1962). Hb-Gower 1 may be a fourth haemoglobin without α -chains, possibly consisting solely of ε -chains (Huehns *et al.*, 1964a).

Huisman (1960) reports the occurrence of a haemoglobin consisting solely of β^S -chains, Hb-Augusta I, and another with only β^C -chains, Hb-Augusta II.

FIG. 9. The amino acid substitutions in the haemoglobin variants modified from Baglioni, 1963a).

(b) **HAEMOGLOBINS CONSISTING SOLELY OF α -CHAINS.** Two haemoglobins of this type have been described: Hb- α^A (Huehns, Shooter, Dance, Beaven, and Shooter, 1961b) and Hb- α^G (Huehns, Dance, Shooter, and Beaven, 1962b). These workers prepared the α -chain haemoglobins by acid dissociation of Hb-A or Hb-F. Since then, Hb- α^A has been detected by starch gel electrophoresis in small amounts in haemolysates from patients with β -thalassaemia major, particularly following splenectomy (Fessas and Loukopoulos, 1964), and from patients with unstable haemoglobins (Huehns and Shooter, unpublished). Hb- α^A cannot be detected in normal haemolysates prepared either from normal adult blood or cord blood by starch gel electrophoresis, though 0.02% of added Hb- α^A can be detected. Chernoff (1964) has isolated trace quantities (0.05–0.3%) of Hb- α^A from normal haemolysates by DEAE column chromatography. However, the finding that when Hb-A₂ or Hb-F are present more Hb- α^A can be detected on re-chromatography of the DEAE column eluate raises the possibility that DEAE promotes the dissociation of some haemoglobins.

(c) **LEPORE HAEMOGLOBIN.** Hb-Lepore was first described by Gerald and Diamond (1958b) in association with the clinical picture of thalassaemia. On starch block electrophoresis Hb-Lepore migrates with Hb-S and amounts to approximately 10–15% of total haemoglobin in the trait form. There are at least two forms of Hb-Lepore. Baglioni (1962b) has shown that these haemoglobins consist of the

N-terminal part of the δ -chain joined to the C-terminal part of the β -chain, the total number of amino acids in the new δ - β -chain being the same as in the β -chain. There are several types of Hb-Lepore, which differ in the proportion of δ - and β -chain present. In Hb-Lepore_{Boston}, obtained from a subject of Italian origin (Gerald and Diamond, 1958b; Baglioni, 1962b), tryptic peptides 1–5 are like those in the δ -chain, and 6–11 could be either from the δ - or β -chain, while 12–15 are β -like. Hb-Lepore_{Hollandia} (Neeb, Beiboer, Jonxis, Kaars Sijpesteijn, and Muller, 1961; Barnabas and Muller, 1962; Baglioni, 1962b) has tryptic peptides 1–3 from the δ -chain and 4–15 from the β -chain (Fig. 10). Several other haemoglobins are probably of the Lepore type: Hb-Pylos (Fessas, Stamatoyannopoulos, and Karakalis, 1962) and Hb-G (Silvestroni and Bianco, 1958). A Hb-Lepore found in a subject of Italian origin has a similar structure to that of Hb-Lepore_{Boston} (Barkhan, Stevenson, Pinker, Dance, and Shooter, 1964).

Baglioni (1962b) has discussed the genetic event that could have led to the formation of a δ - β -polypeptide chain. Two possibilities are mentioned, both of which require the *Hb β* and *Hb δ* loci to be closely linked (see later). The first possibility is a deletion of part of the δ and β loci with the formation of a new δ - β gene. On this hypothesis it is difficult to understand why the δ - β -chain synthesized is the same length as the β - or δ -chain. The second, and apparently more likely, situation, which accounts for the

FIG. 10. Schematic representation of the Lepore_{Boston} gene (top), of the Lepore_{Hollandia} gene (bottom), and of the corresponding peptide chains. The shaded areas indicate that part of the Lepore genes which appears to be derived from the Hb δ gene and that part of the corresponding peptide chains which is δ -like. The area in solid colour indicates that part of the Lepore genes which appears to be derived from the Hb β gene and that part of the corresponding peptide chains which is β -like. The empty areas between shaded and solid colour areas indicate those portions of the genes and of the peptide chains that may be derived from either the Hb δ or Hb β gene. The postulated joining of part of the Hb δ gene to part of the Hb β gene has occurred presumably in this area. The lines along the peptide chains indicate schematically the peptide bonds that may be split by trypsin. The resulting peptides are indicated below and are numbered consecutively starting from the N-terminus. The symbols δ or β which precede the peptide numbers indicate whether the corresponding peptides are δ -like or β -like. (From Baglioni, 1962b, with permission of National Academy of Sciences.)

joining of the β to the δ -gene in such an exactly complementary way, is a non-homologous crossing-over between corresponding points of the β and the δ genes, resulting in the formation of unequal products, one of which is Hb-Lepore (Fig. 11).

FIG. 11. Schematic representation of the postulated non-homologous crossing-over leading to the formation of the Lepore genes. The shaded area indicates the nucleotide sequence corresponding to the Hb_S gene. The non-homologous crossing-over is supposed to have occurred in the areas shown as empty in Fig. 10. (From Baglioni, 1962b, with permission of National Academy of Sciences.)

Methods for the Identification of the Abnormal Haemoglobins

(i) **ELECTROPHORESIS.** This is the primary method for the detection of any haemoglobin variant. Originally, moving boundary electrophoresis was used, but this was soon replaced by paper electrophoresis, and this method is the most widely used technique. However, a number of the haemoglobins that are associated with disease, such as Hb-Lepore, Hb-St. Mary's, Hb-H, and Hb- γ_4 , are not easily detected by this procedure, particularly as they are often present in low concentrations, while variants of Hb-A₂ cannot be detected at all. Starch gel electrophoresis has a much greater sensitivity and is now the method of choice. Suitable buffer systems have been previously described (Gammack, Huehns, Shooter, and Gerald, 1960), but the greatest over-all sensitivity can be achieved using a *tris*-E.D.T.A.-borate buffer system (O. Smithies, unpublished). This is sensitive both to the rapidly migrating haemoglobins such as the non- α -chain haemoglobins, Hb- β_4 and Hb- γ_4 , as well as to 'slow' minor components. The usual pH used is 8.6 (109 g. *tris* [2-amino-2-(hydroxy-methyl) propane-1:3 diol] 5.84 g. disodium E.D.T.A., 30.9 g. boric acid/l., diluted 1 to 20 for making the gel and 1 to 7 for the buffer vessels). One of the advantages of this system is that concentrated haemolysates can be applied without overloading the gel, thus facilitating the detection of minor components. The sensitivity can be further increased by staining the gel with benzidine or *o*-tolidine. Another advantage is that 1% of Hb-F can be detected. In order to detect the presence of Hb- α^A , which remains on

the origin in this system at pH 8.6, the gel can be made at pH 8.3, using 69.9 g. *tris*/litre instead of 109 g.

The relative concentration of Hb-A₂, which is of some importance in the diagnosis of thalassaemia trait, is best estimated by starch block electrophoresis (Kunkel, 1954). It can also be estimated by column chromatography on DEAE (Huisman and Dozy, 1962). In practice, these methods are time consuming and open to error if not carried out carefully. An estimate to determine whether Hb-A₂ is raised can be obtained by careful comparison of the Hb-A₂ zones obtained on starch gel electrophoresis from normal haemolysates with those from suspected thalassaemia minor after adjustment of the haemoglobin concentration in both.

Agar gel is also a good supporting medium for electrophoresis of haemoglobins (Yakulis, Heller, Josephson, and Singer, 1960; Shibata and Iuchi, 1962) and gives some special separations when used at acid pH (Robinson *et al.*, 1957).

(ii) **CHROMATOGRAPHY.** This method is mainly used for the purification of haemoglobins before chemical analysis and has been carried out on IRC 50 (Allen *et al.*, 1958; Clegg and Schroeder, 1959; Schneck and Schroeder, 1961; Jones and Schroeder, 1963), carboxymethylcellulose (CMC) (Huisman *et al.*, 1958) or diethylaminoethylcellulose (DEAE) (Huisman and Dozy, 1962). Huisman and Prins (1955) have described a qualitative method which has often been used for the identification of haemoglobins (see Lehmann, 1960).

(iii) **HYBRIDIZATION.** This method, first devised by Itano and his co-workers (Itano and Singer, 1958; Itano, Singer, and Robinson, 1959), is used in the identification of the abnormal chains of a haemoglobin variant. A suitable micro-adaptation of the original method, using starch gel for the analysis of the haemoglobins formed, has been described (Gammack *et al.*, 1960). More recently the formation of hybrid haemoglobins between human and canine haemoglobins has been used (see Fig. 12) (Itano and Robinson, 1959; Robinson and Itano, 1960; Huehns, Shooter, and Beaven, 1962a; Shibata, Iuchi, Ueda, Miyaji, and Takeda, 1962b).

(iv) **'FINGERPRINTING'.** This method is used to identify the abnormal tryptic peptide and is fully described by Ingram (1958) and Baglioni (1961). The globin of the purified abnormal haemoglobin is prepared (Anson and Mirsky, 1930; Rossi-

FIG. 12. The formation of hybrid haemoglobins between Hb-A and canine haemoglobin and between Hb-G and canine haemoglobin: (i) Hb-A + Hb-Canine control; (ii) Hb-A + Hb-Canine dissociated and recombined; (iii) Hb-G + Hb-Canine dissociated and recombined; and (iv) Hb-G + Hb-Canine control. Since Hb-G has the structure $\alpha_2^G\beta_2^A$, it forms the same hybrid $\alpha_2^{Can}\beta_2^A$, as does Hb-A, but the second hybrid $\alpha_2^G\beta_2^{Can}$ is different from $\alpha_2^A\beta_2^{Can}$. This method can be used to find which chain is abnormal in a haemoglobin variant (from Huehns and Shooter, 1964a; with kind permission of Blackwell Scientific Publications Ltd.).

Fanelli, Antonini, and Caputo, 1958; Teale, 1959) and digested with trypsin. The resulting peptide mixture is then analysed by high-voltage electrophoresis, followed by chromatography at right angles. The resulting peptide pattern or 'fingerprint' is compared with that prepared from normal haemoglobin (Fig. 13). The abnormal peptide is then eluted and the amino acid sequence determined. One of the drawbacks of this method is that some of the peptides formed are insoluble, the so-called 'core'. Recently, Jones (1964) has aminoethylated the cysteinyl residues in haemoglobin by treatment of the globins with ethylenimine at pH 8.6 in 8M urea. The S-(2-aminoethyl)-cysteinyl ('pseudo-lysine') residues formed lead to several more sites in the 'core' of the molecule, which are cleaved by trypsin, and thus no insoluble peptides are formed. The peptide mixture formed can then be conveniently separated by automatic column chromatography.

One of the questions in the examination of the abnormal haemoglobin is how far the investigation of each separate sample containing a haemoglobin variant should be taken. From the clinical point of view, only a limited amount of work is necessary. The ability of the red cells to sickle on reduction

with metabisulphite would distinguish between the presence of Hb-D and Hb-S. The identification of some of the other abnormal haemoglobins, such as Hb-Lepore, Hb-H, Hb-M, and the unstable haemoglobins, is suggested by the associated clinical picture. For the remaining haemoglobins, the identification is often presumptive, depending on careful comparison with known markers. The relative proportion of the abnormal haemoglobin found in the heterozygote and the origin of the patient are both taken into consideration. From the biochemical geneticist's point of view, no identification is complete until the abnormal amino acid residue has been located. Even haemoglobins apparently identical by electrophoresis and chromatography as well as by hybridization are found to be different by fingerprinting, for example, the various types of Hb-D and Hb-J mentioned earlier. On the other hand, an identical fingerprint is found in Hb-J_{Rambam} and Hb-Seattle. One of these has a glycine residue replaced by aspartic acid in tryptic peptide IX, while in the other an alanine is replaced by glutamic acid in the same peptide. In practice, rare variants found in ethnically different populations probably merit detailed study.

FIG. 13. The fingerprints of Hb-A and the variant Hb-Seattle. The peptides were stained by spraying with ninhydrin. Electrophoresis in pyridine acetic acid buffer pH 6.4 followed by chromatography in *iso*-amyl alcohol-pyridine-water solvent. (Photographs supplied by Mr N. Dance.) (From Huehns and Shooter, 1964a; with kind permission of Blackwell Scientific Publications Ltd.)

(v) SEPARATION OF THE POLYPEPTIDE CHAINS OF Hb-A. The peptide analyses described above are very much simplified if the α - and β -chains are first separated. This can be achieved by column chromatography on Amberlite IRC 50 in a urea gradient at pH 2 (Wilson and Smith, 1959) or by countercurrent distribution (Hill and Craig, 1959). Hayashi (1961) has separated the chains by differential precipitation. The globin chains can also be obtained by starch gel electrophoresis (Muller, 1960). It is much more difficult to separate the polypeptide chains with their haem groups attached, and chromatography at acid pH gives only small yields (Huehns *et al.*, 1961b). More recently, striking separations of the α - and β -chains of Hb-A with their haem groups still attached have been obtained after reacting the haemoglobin with an excess of sodium *para*-(Chloromercuri) benzoate (PCMB) in the presence of 0.2M-NaCl at pH 6.0 (R. Bucci and C. Frontacelli, unpublished).

Other Biochemical Studies

(i) IMMUNOLOGICAL STUDIES OF HUMAN HAEMOGLOBINS. Immunological studies on haemoglobin

have been reported, and antibodies to Hb-A, Hb-F, and Hb-A₂ have been prepared (Chernoff, 1953a, Westendorp Boerma, Huisman, and Mandema, 1960; Heller, Yakulis, and Josephson, 1962a; Schneider and Arat, 1964; Askonas and Smyth, 1964). Rucknagel and Chernoff (1955) have used anti-Hb-F to measure small amounts of Hb-F in normal adults and during pregnancy.

Recently, Westendorp Boerma and Huisman (1964) have produced antisera in rabbits which, after suitable absorption procedures, can differentiate between Hb-S and Hb-C, a remarkable degree of specificity when it is remembered that these haemoglobins only differ by one amino acid on each of the β -chains. Another interesting observation is that there is an immunological difference between horse oxy- and deoxyhaemoglobin (Reichlin, Bucci, Antonini, Wyman, and Rossi-Fanelli, 1964). This observation provides further evidence that oxyhaemoglobin has a different conformation from deoxyhaemoglobin, as has already been shown by the studies of Muirhead and Perutz (1963) referred to above.

(ii) THE SITE OF CHROMIUM ATTACHMENT TO HAEMOGLOBIN. Radioactive chromium (^{51}Cr) is commonly used to label red cells for *in vivo* cell survival studies. It is known that it is attached to the haemoglobin molecule (Gray and Sterling, 1950). Experiments by Chernoff (1961) suggest that the site of attachment might be on the α -chain. However, the work of several other investigators (Pearson and Vertrees, 1961; Heisterkamp and Ebaugh, 1962; Malcolm, Ranney and Jacobs, 1963) makes it more likely that it is mainly attached to the β -chain. Perhaps the method of labelling red cells with ^{51}Cr is critical.

II: THE GENETIC CONTROL OF HAEMOGLOBIN SYNTHESIS

Structural Loci

Adult Haemoglobin. In 1949, Pauling *et al.* showed that the haemoglobin of patients with sickle cell disease differed from that of normal persons and their studies indicated that,

'two normal genes lead to the production of only normal haemoglobin; one normal and one abnormal gene lead to the production of both normal and sickle haemoglobin and two abnormal genes lead to the production of only sickle haemoglobin'.

At about the same time, the family studies of Beet (1949) and of Neel (1949), using the sickling

FIG. 14. Pedigree of 'Hopkins 2 pedigree' showing independent segregation of the genes for Hb-S and Hb-Hopkins 2 (from Smith and Torbert, 1958; and Bradley *et al.*, 1961).

test, also demonstrated the inheritance of the disease. Shortly after this, a number of abnormal haemoglobins (Hb-C, Hb-D, Hb-E, etc.) were discovered which showed similar inheritance, and Itano (1953) suggested that because individuals with both Hb-C and Hb-S carried no Hb-A, the genes responsible for these two haemoglobins were alleles. This was shortly afterwards confirmed by the family studies of Ranney (1954). A similar allelism was proposed for Hb-D, Hb-E, and Hb-G (Neel, 1956). This rests mainly on the finding that double heterozygotes carry no Hb-A, rather than on classical genetic analysis. The discovery by Smith and Torbert (1958) of a family where two abnormal haemoglobins, Hb-S and Hb-Hopkins 2, were segregating independently, and the finding that one had abnormal β -chains while the other had abnormal α -chains, led to the present concept of the genetic control of haemoglobin synthesis (Itano *et al.*, 1959).

Because the abnormal haemoglobins have either abnormal α -chains or abnormal β -chains, it was suggested that these were controlled by separate genetic loci (Itano and Robinson, 1959; Ingram, 1959). Studies of several families (see Fig. 14) (Smith and Torbert, 1958; Itano *et al.*, 1959; Atwater, Schwartz, and Tocantins, 1960b; Baglioni and Ingram, 1961; Raper, Gammack, Huehns, and Shooter, 1960) in which both α -chain and β -chain variants were segregating and in which some individuals had inherited both abnormalities showed that the locus controlling the synthesis of the α -

chains, the so-called Hb α locus, was not linked to that controlling the β -chain, the so-called Hb β locus. Furthermore, those individuals that had inherited both abnormalities carried not only both abnormal haemoglobins but also Hb-A and a fourth haemoglobin with both abnormal α - and β -chains (Raper *et al.*, 1960; Baglioni and Ingram, 1961). On the basis of all these findings, a hypothesis for the genetic control of synthesis of Hb-A was developed (Itano and Robinson, 1960; Raper *et al.*, 1960; Baglioni and Ingram, 1961).

The synthesis of the α - and β -chains of Hb-A is controlled by pairs of genes at different genetic loci, and the genotype can conveniently be written $Hb^A_\alpha Hb^A_\alpha Hb^A_\beta Hb^A_\beta$ (the superscript *A* indicating that the gene is controlling the synthesis of a normal polypeptide chain). Each gene at each locus indirectly determines the synthesis of a particular type of polypeptide chain. These then associate to form the haemoglobins found *in vivo* (Fig. 15).

Those individuals who inherit one abnormal gene will make two haemoglobins (Fig. 16).

If a person inherits two abnormal genes, three different situations can arise.

(i) A person can inherit two abnormal genes of the same type, as, for example, in the genotype $Hb^A_\alpha Hb^A_\alpha Hb^C_\beta/Hb^C_\beta$. In these subjects, only α^A - and β^C -chains are formed, and only Hb-C ($\alpha^A_2\beta^C_2$) is found in the red cells. The inheritance of two genes of the same type usually leads to a

FIG. 15. The genetic control of the synthesis of haemoglobin A in a normal individual (from Huehns and Shooter, 1964a).

FIG. 16. The genetic control of the synthesis of haemoglobin in a sickle cell heterozygote (from Huehns and Shooter, 1964a).

mild haemolytic disease, such as Hb-C disease, Hb-E disease, etc.

(ii) When two different abnormal genes occur at the same *locus*, as, for example, in the genotype $Hb_{\alpha}^A/Hb_{\alpha}^A$ $Hb_{\beta}^S/Hb_{\beta}^C$, α^A -, β^S -, and β^C -chains are made, leading to the formation of only Hb-S and Hb-C.

(iii) Finally, if two different mutations occur, one

at each locus, as, for example, in the individuals with four adult haemoglobins mentioned earlier, four different polypeptide chains are synthesized, leading to the formation of four haemoglobin species (Fig. 17).

In this connexion, it is of interest that individuals with clinically severe sickle cell anaemia have been reported who carry two abnormal haemoglobins

FIG. 17. The genetic control of the synthesis of haemoglobin in an individual heterozygous for the Hb-C trait and the Hb-G α trait (from Huehns and Shooter, 1964a).

FIG. 18. The genetic control of the synthesis of normal adult haemoglobins, Hb-A and Hb-A₂, foetal haemoglobin, and the embryonic haemoglobins, Hb-Gower 1 and Hb-Gower 2 (from Huehns and Shooter, 1964a).

(Pugh, Monical, and Minnich, 1964; Hall-Craggs, Marsden, Raper, Lehmann, and Beale, 1964). In these individuals there is homozygosity at the Hb_{β} locus for the β^s gene but heterozygosity at the Hb_{α} locus, giving the genotype $Hb_{\alpha}^A/Hb_{\alpha}^G Hb_{\beta}^s/Hb_{\beta}^s$. Clearly, two haemoglobins, Hb-S ($\alpha_2^G\beta_2^s$) and Hb-G S ($\alpha_2^A\beta_2^s$) will be made, and, as both contain β^s -chains, the patient clinically has sickle cell disease.

Haemoglobins A₂ and F. The genetic control of synthesis of Hb-A₂ and Hb-F follows the same pattern as that of Hb-A. As the α -chains of the normal human haemoglobin are all identical, it has been suggested that these arise from a common metabolic pool and thus are controlled by the same genetic locus (Huehns and Shooter, 1961a). The non- α -chains of Hb-A₂ and Hb-F, the δ - and γ -chains respectively, are controlled by their own genetic loci, the Hb_{δ} and Hb_{γ} loci. Although no genetic studies on the embryonic haemoglobins have been possible, it is postulated that there is a separate genetic locus for the ϵ -chain. The outline for the control of synthesis of the normal haemoglobins would then be as in Fig. 18. The above hypothesis is supported by the discovery of variants of δ - and γ -chains mentioned earlier. The idea that the α -chains of Hb-F, Hb-A₂, and Hb-A arise from a common metabolic pool implies that if abnormal α -chains are being synthesized, not only will a Hb-A variant be found but also the corres-

ponding variants of Hb-F and Hb-A₂ with abnormal α -chains (Fig. 19).

Studies of several people synthesizing abnormal α -chains have, in fact, shown that they carry not only the corresponding Hb-A variant but also the corresponding variant of Hb-A₂: Hb-G₂ (Shooter, Skinner, Garlick, and Barnicot, 1960; Huehns and Shooter, 1961a, b), Hb-I₂ (Atwater, Huehns, and Shooter, 1961; Boulard, Cosset, Destaing, Duzer, Jonxis, Muller, and Portier, 1961), Hb-Q₂ (Dormandy, Lock, and Lehmann, 1961), Hb-No₂ (Baglioni, 1962c), Hb-D₂ and Hb-G₂ (Weatherall and Boyer, 1962) and Hb-F: Hb-G₂F and Hb-D₂F (Minnich *et al.*, 1962; Weatherall and Boyer, 1962; Weatherall and Baglioni, 1962), Hb-I₂F (Ranney, O'Brien, and Jacobs, 1962).

'Controller Genes' in Haemoglobin Synthesis

During development, the synthesis of embryonic haemoglobin is succeeded by the synthesis of foetal haemoglobin which, in turn, is replaced by the adult haemoglobins, Hb-A and Hb-A₂, shortly after birth. This can be explained in terms of regulatory genes which control the rates of synthesis of various polypeptide chains, a concept derived from work in bacterial genetics (Jacob and Monod, 1961). A mutation at one of these controller loci might result in changes in the rate of synthesis of a polypeptide chain without any alteration in its structure, and it has been suggested that 'the hereditary persistence

FIG. 19. The genetic control of haemoglobin in a subject heterozygous for Hb-G α trait, showing the formation of the three normal haemoglobins Hb-A, Hb-F, and Hb-A₂, as well as the corresponding α -chain variants Hb-G, Hb-G/F, and Hb-G₂.

of foetal haemoglobin' and 'thalassaemia' are of this type (Neel, 1961; Motulsky, 1962; Sturgeon, Schroeder, Jones, and Bergren, 1963; Zuckerkandl, 1964).

Linkage Relationships of the Loci Controlling the Synthesis of Haemoglobin

(i) **Hb α and Hb β Loci.** Studies of several families in which both α - and β -chain variants of Hb-A are segregating (quoted above) show that the locus controlling the synthesis of the α -chains is not linked to that of the β -chains. It has also been shown that neither loci are closely linked to genetic loci controlling certain blood or serum groups (Bradley, Brawner, and Conley, 1961).

(ii) **Hb β and Hb δ Loci.** There are several families in which a Hb-A₂ variant with abnormal δ -chains segregates with a β -chain variant and no cross-overs have been detected, though this could have taken place in 45 instances (Ceppellini, 1959; Horton *et al.*, 1961; Ranney *et al.*, 1963; Boyer Rucknagel, Weatherall, and Watson-Williams, 1963; Horton and Huisman, 1963). These family studies thus indicate that the locus controlling the synthesis of the δ -chain is closely linked to that for the β -chain. The family study of Huisman, Punt, and Schaad (1961) showed that the locus for δ -chains was linked to that for β -thalassaemia, and as the latter was linked or allelic to that controlling the synthesis of β -chains (see below), it also strengthened the finding of δ to β linkage.

(iii) **Hb β and Hb γ Loci.** At the present time, no studies of families in which variants of both the γ - and β -chains occur have been reported, and the question of whether the locus controlling the synthesis of the γ -chains is linked to the loci controlling the synthesis of β - and δ -chains cannot be definitely answered. However, studies of families in which the β -chain variants Hb-S or Hb-C are segregating with a gene causing persistence of foetal haemoglobin, the so-called 'high-F gene', indicate that the 'high-F' gene is closely linked (or allelic) to the β -chain locus (Rucknagel and Neel, 1961; Conley, Weatherall, Richardson, Shepard, and Charache, 1963). At present, it seems reasonable to assume that the 'high-F' gene might be at, or closely linked to, the locus for the γ -chains, and this leads to the conclusion that Hb γ and Hb β loci are closely linked.

(iv) **Linkage Relationships of the Thalassaemia Loci.** The results of studies of families in which both β -thalassaemia (see later) and haemoglobins S, C, or B₂ are segregating have been reviewed by Rucknagel and Neel (1961) and Motulsky (1964b) and indicate that the gene causing β -thalassaemia is closely linked or allelic to the β and δ structural loci. There appears to be only one clear instance of a cross-over between the β -thalassaemia gene and that for an abnormal haemoglobin, in this case, Hb-B₂ (Moore and Pearson, 1964). Motulsky (1964b) tentatively interprets this finding as evidence in support of the idea that the locus for β -thalassaemia is outside the $\beta\delta$ -structural

locus but closely linked to it. Clearly, further family studies to resolve this point are needed.

The α -thalassaemia locus, like the Hb α structural locus, is not linked to the Hb β structural locus (Tuchinda, Rucknagel, Minnich, Boonyaparakob, Balankura, and Suvatee, 1964). There are no data yet to show whether α -thalassaemia trait is linked to the Hb α structural locus. The biochemical interaction between α -thalassaemia and α -chain variants of haemoglobin is similar to that between β -thalassaemia and the various β -chain haemoglobin variants, and this suggests that the genetical situation might be analogous to that for β -thalassaemia.

The Problem of the Inheritance of Haemoglobin M

Although, in several families, Hb-M follows the semi-dominant pattern of Mendelian inheritance seen with other abnormal haemoglobins, in several instances the disease is not found in either of the parents of one of the affected individuals. Three families (Sahawi, Hunger, and Betke, 1962; Josephson, Weinstein, Yakulis, Singer, and Heller, 1962; Heller, Weinstein, Yakulis, and Rosenthal, 1962b) like this have now been studied in detail, and the determination of a number of blood group characters in various members of the families is consistent with the stated parentage (Sahawi *et al.*, 1962; Josephson *et al.*, 1962). This could be explained if the formation of methaemoglobin *in vivo* in these individuals only occurred by the interaction of the haemoglobin abnormality with some other, as yet undiscovered, factor. This explanation is ruled out by the absence of any haemoglobin abnormality in detailed studies of the unaffected family members. It would then appear that the occurrence of Hb-M in these individuals is due to new mutations.

Haemoglobins and Chromosomal Abnormalities

It appears firmly established, as outlined in the preceding section, that haemoglobin synthesis is controlled by a number of genetic loci, one structural locus for each of the different polypeptide chains. There are probably also a number of regulatory loci. One of the most intriguing questions is on which of the 46 chromosomes these loci are situated. The inheritance of the abnormal haemoglobins indicates that they are not X-linked. Recently, the haemoglobins from patients with the various chromosomal triplications, the so-called trisomies, have been investigated. Huehns, Hecht, Keil, and Motulsky (1964c) and Huehns *et al.*

(1964a, b) report that, in trisomy 13-15, trace amounts of the embryonic haemoglobin Hb-Gower 2 persist until birth and that Hb- γ_4 is present in slightly increased amounts. They also found that in six older children with the same trisomy, Hb-F was increased. The data on the Hb-F in these individuals are still very scanty, but it appears that its disappearance is delayed as compared to that of normal individuals. Powars, Rohde, and Graves (1964) have also found persistence of Hb-F in their patient with trisomy 13-15. These results can be interpreted in several ways, but raise the possibility that some of the loci controlling haemoglobin synthesis are located on the chromosome triplicated in this syndrome. However, another view is that these findings represent a more generalized immaturity in biochemical development. The haemoglobins found in trisomy 17-18 and trisomy 21-22 (Down's syndrome) have also been studied. Huehns *et al.* (1964c) report no difference from normal in both conditions (one patient with trisomy 17-18 and six with trisomy 21-22). D. L. Rucknagel (personal communication) has found that in 10% of Negroes with Down's syndrome, Hb-F is increased (90 cases studied).

Further information will be obtained if similar patients can be found who also have an abnormal haemoglobin, since the ratio of normal to abnormal haemoglobin might differ if the triplicated chromosome carries one or more of the loci controlling haemoglobin synthesis. D. L. Rucknagel (personal communication) has studied the Hb-A to Hb-S ratios in patients with trisomy 21-22 and found them to be normal. Of course, if a patient with one of the trisomies carries, say, Hb-S, Hb-C, and Hb-A, he must then have inherited three different Hb β structural genes, one on each of the triplicated chromosomes. Although the combination of a mating of a patient with Hb-S-C disease with a normal person giving rise to an infant with one of the trisomies must be extremely rare, the search for and detailed study of such patients may lead to further advances in this interesting field.

The Abnormal Haemoglobins and the Genetic Code

In the genetic control of protein synthesis, it is postulated that the amino acid sequence of any protein is represented on the chromosome by a definitive sequence of bases in the DNA. Crick, Barnett, Brenner, and Watts-Tobin (1961), from their genetic experiments in bacteria, have suggested that each amino acid is coded for by three (or perhaps a multiple of three) bases and that each

'code word' is specific for one amino acid. The different code words are non-overlapping and follow directly one after another. It appears that the code is 'degenerate', that is, there may be more than one code word for any particular amino acid. In order that synthesis of protein can take place, the DNA code is transcribed on to the so-called messenger RNA (mRNA) which is thought to form the template on the ribosomes on which the protein is synthesized. Each amino acid is added at the right place because of the complementary fit of the amino acid transfer RNA. This hypothesis implies that the amino acid sequence of a protein is co-linear with the nucleotide sequence in the corresponding gene (Sarabhai, Stretton, Brenner, and Bolle, 1964; Yanofsky, Carlton, Guest, Helinski, and Henning, 1964). The triplet 'code letters' which probably code for each amino acid on the mRNA have been the subject of much investigation (Nirenberg, Jones, Leder, Clark, Sly, and Pestka, 1963). A current summary of the proposed RNA code words is given in Table III. It can be

valine in Hb-S and glutamic acid → lysine in Hb-C fit in as shown below.

III: THE BIOSYNTHESIS OF HAEMOGLOBIN

It has been shown that the synthesis of the α - and β -chains of Hb-A are controlled by separate, unlinked genes, and that in individuals carrying both abnormal α -chains and abnormal β -chains four types of haemoglobins are found (Fig. 17). It can thus be assumed that the α -chains are synthesized separately from the β -chains. From the work of Bishop, Leahy, and Schweet (1960) and Dintzis (1961), it is known that synthesis of the polypeptide chains begins at their N-terminals and the amino acids are added sequentially until the chains are completed. The haem groups are separately synthesized (see Rimington, 1959). Following synthesis, the polypeptide chains and haem groups are assembled into the tetramer haemoglobins found in the red cell. At present, it is not known at which stage the haem groups are attached, but there is some knowledge of the order in which the polypeptide chains assemble. This comes from recent experiments on the dissociation of haemoglobin.

The Dissociation of Haemoglobin

It is well known that haemoglobin dissociates at acid pH and that in mixtures of suitable haemoglobins exchange of chains takes place with the formation of hybrid haemoglobins. A good illustration is the formation of hybrids between human and canine haemoglobins (Robinson and Itano, 1960; Huehns *et al.*, 1962a) (Fig. 12). The composition of the hybrids formed is given by the following over-all reaction:

Some of each of the parent species are also reformed. Although this result may be explained most readily by postulating that each haemoglobin dissociates into α_2 and β_2 subunits and that these subunits then exchange, there are a number of observations

TABLE III

SUMMARY OF RNA CODE WORDS (Nirenberg *et al.*, 1963)

Amino Acid	RNA Code Words*			
Alanine	CCG	UCG†	ACG†	CGA†
Arginine	CGC	AGA	UGC†	
Asparagine	ACA	AUA	ACU†	
Aspartic acid	GUA	GCA†	GAA†	
Cysteine	UUG			
Glutamic acid	GAA	GAU†	GAC†	
Glutamine	AAC	AGA	AGU†	
Glycine	UGG	AGG	CGG	
Histidine	ACC	ACU†		
Isoleucine	UAU	UAA		
Leucine	UUG	UUC	UCC	UUA
Lysine	AAA	AAU		
Methionine	UGA			
Phenylalanine	UUU	CUU		
Proline	CCC	CCU	CCA	CCG†
Serine	UCU	UCC	UCG†	ACG
Threonine	CAC	CAA		
Tryptophan	GGU			
Tyrosine	AUU			
Valine	UGU	UGA†		

* Arbitrary nucleotide sequence.

† Probable.

seen that more than one 'code word' has been proposed for some amino acids, in keeping with the suggested 'degeneracy' of the genetic code. It has been postulated that a point mutation is a change of a single nucleotide base in a three-letter code word and all the known amino acid substitutions in the abnormal haemoglobins are consistent with this hypothesis. For example, the two substitutions at position 6 of the β^A -chain, glutamic acid →

that do not fit into this scheme. For example, it is known that both Hb-A and canine haemoglobin dissociate into subunits immediately the pH is lowered below 5.0. Yet it is necessary to hold the two haemoglobins at these pH values for many hours before exchange of subunits and formation of hybrids occur (Labie, Rosa, Dreyfus, and Shapira, 1962; Huehns, Shooter, and Beaven, 1964d). Clearly if the subunits were α_2 and β_2 , there would be no reason for this delay in exchange. However, it can be accounted for if it is assumed that the initial rapid dissociation is into $\alpha\beta$ subunits, that these further dissociate at a slower rate into single α - and β -chains, and that the exchange takes place at the single chain level (Vinograd and Hutchinson, 1960). Supporting evidence for this mechanism comes from the finding that the isolated α^A -chain subunits from Hb-A do, in fact, exist in solution as stable monomers rather than α_2^A dimers (Huehns *et al.*, 1961b). The question then arises as to why only like $\alpha\beta$ subunits unite, since species with unlike α - or unlike β -chains have not been observed. Moreover, attempts to produce such species by a number of different experimental approaches have failed (Huehns and Shooter, 1964b). For example, globins with the structure $\alpha\beta$ have been prepared which reform tetramer haemoglobins on the addition of haem (Winterhalter and Huehns, 1964). When a mixture of globin C is mixed with globin A and haem added, three haemoglobin species could form, as follows.

However, on starch gel analysis of the reacted product, only Hb-A and Hb-C were detected. What then prevents an $\alpha^A\beta^A$ subunit combining with an $\alpha^A\beta^C$ subunit to form the hybrid $\alpha_2^A\beta^A\beta^C$? One explanation (Vinograd and Hutchinson, 1960) is that recombination is specific and that only like $\alpha\beta$ subunits can combine with one another. This explanation seems unlikely, since it is very difficult

to see how a single amino acid substitution on the surface of the coiled polypeptide chain could prevent association of an $\alpha^A\beta^A$ subunit with an $\alpha^A\beta^C$ subunit. Moreover, all the single amino acid substitutions in the many abnormal haemoglobins would have to obey the same restriction. Recently Guidotti, Konigsberg, and Craig (1963) have put forward a plausible explanation. They suggest that such mixed hybrids with unlike α - and unlike β -chains do form, and that there is no specificity in the recombination of $\alpha\beta$ subunits. They argue that the reason why these species are not observed is because even at neutral pH haemoglobin is dissociated (Drabkin and Wise, 1962; Huehns, 1962; Huehns, Beaven, and Stevens, 1964f) and is in equilibrium with $\alpha\beta$ dimers, i.e. $\alpha_2\beta_2 \rightleftharpoons 2\alpha\beta$ (Huehns, 1962). It follows that in any separation process the species $\alpha_2^A\beta^A\beta^C$, for example, will dissociate into $\alpha^A\beta^A$ and $\alpha^A\beta^C$ subunits and that these will then predominantly form the species $\alpha_2^A\beta_2^A$ and $\alpha_2^A\beta_2^C$. This hypothesis eliminates the need to postulate steric factors to prevent unlike $\alpha\beta$ subunits combining.

There are two haemoglobin species in which unlike chains occur, Hb-F₁ ($\alpha_2^A\gamma^F\gamma^{AcF}$) (Schroeder *et al.*, 1962) and Hb-A_{1c} ($\alpha_2^A\beta^A\beta^X$) (Holmquist and Schroeder, 1964). In Hb-F₁, the N-terminal of one of the γ -chains is blocked by an acetyl group, while in Hb-A_{1c} the N-terminal of one of the β -chains is blocked by an unidentified grouping. If the above explanations prove correct, then some special explanation for the ability to isolate these two mixed species must be found and this is discussed elsewhere (Huehns and Shooter, 1964b).

It does not necessarily follow that the assembly of the haemoglobin molecule will maintain the same course as its dissociation, but the properties of the isolated subunits, in particular, the stability of the monomer α^A -chain subunit, as well as the close contact between α - and β -chain subunits demonstrated by the x-ray work, make this the most plausible mechanism.

Since, in the normal adult, equal numbers of α - and β -chains are produced, there is presumably a mechanism adjusting the rate of synthesis of one of the chains to that of the other. In deficiency of α -chain synthesis— α -thalassaemia—Hb- β_4 is found in quantity (see later), while in the severest deficiencies of β -chain synthesis— β -thalassaemia major—only trace amounts of free α -chains are found (Fessas and Loukopoulou, 1964). These findings have led to the suggestion that completed β -chains are necessary for the free release of α -chains from their site of synthesis (Huehns and Shooter, 1962). In this way, the effective rate of α -chain synthesis

is adjusted to that of β -chains. In release of the completed chains, an $\alpha\beta$ subunit is formed, and finally two of these combine to form the tetramer haemoglobin molecules found in the red cell. The haem group is separately synthesized. It appears that the rate of haem synthesis is linked to that of globin synthesis by a feedback mechanism, any excess haem formed depressing the activity of δ -amino-laevulinic acid (δ -ALA) synthetase (Lascelles, 1960; Burnham and Lascelles, 1963).

It is not clear yet at which stage the haem group combines with the globin chains. The finding that there is a small amount of free globin in the red cells (Winterhalter and Huehns, 1963) leads to the speculation that the $\alpha\beta$ intermediate postulated above is globin (which is known to have the structure $\alpha\beta$) and that the addition of haem brings about the association of two $\alpha\beta$ subunits.

IV: CLINICAL FEATURES OF ABNORMALITIES IN HAEMOGLOBIN SYNTHESIS

The various ways in which abnormalities of protein synthesis might cause disease are well demonstrated by the abnormalities in haemoglobin synthesis. It can be envisaged that disease can be caused in several ways:

- (i) the production of an abnormal protein molecule—the haemoglobinopathies;
- (ii) the reduction in the amount of *normal* protein synthesized—the thalassaemias;
- (iii) developmental anomalies—the persistence of foetal haemoglobins.

The Haemoglobinopathies

The haemoglobinopathies can be divided into several groups. Clinically, the most important of these is sickle cell disease and its variants, and this disease process is brought about by a specific new property of the protein consequent to the amino acid substitution. The haemoglobin M diseases form another group, in which the ability to carry oxygen is due to the *in vivo* formation of abnormal methaemoglobins. Other haemoglobinopathies are characterized by the presence of haemolytic anaemia associated with the presence of unstable abnormal haemoglobins in the heterozygous state. Finally, there are the mild diseases that occur when some abnormal haemoglobin, such as Hb-C, Hb-D, or Hb-E, is found in the homozygous state.

(i) **Sickle Cell Disease.** The clinical features of this disease and the milder syndromes caused by the interaction with other haemoglobin syndromes are already well described (Wintrobe, 1961). However, in recent years, the way in which the substitution of a single amino acid (glutamic to valine) in each of the β -chains leads to this severe disease has now been delineated by Murayama (1962b, 1964). He has suggested that in Hb-S the substitution of a glutamic residue at position 6 by a valyl residue leads to the formation of a ring of amino acids at the N-terminus of each of the β -chains, the N-terminal valine forming a non-covalent (hydrophobic) bond with the genetically introduced valine at position 6. These cyclic structures on each of the β -chains are situated in such a position that when the haemoglobin molecule is reduced, they interlock with the α -chains of the next haemoglobin molecule, forming long chains. These aggregates are presumably birefringent spindle-shaped bodies, 1–15 μ in length, the so-called tactoids (Sherman, 1940), which characteristically occur in sickle cells. The fact that the *in vitro* and *in vivo* formation of sickle cells depends on the oxygen tension is well known (Hahn and Gillespie, 1927; Lange, Minnich, and Moore, 1951; Harris, Brewster, Ham, and Castle, 1956) and fits in well with Murayama's ideas. Muirhead and Perutz (1963) have shown that the β -chains of reduced haemoglobin are 7 Å further apart than in the oxygenated form, while the α -chains show no such change in position. Clearly, if a 'key' on each of the β -chains can fit into the α -chains of the next molecule in the reduced state, this fit will not be possible in the oxygenated state when the β -chains in the molecule are closer together.

The sickling of the erythrocyte causes two significant changes. First, it causes an increase in the mechanical fragility (Diggs and Bibb, 1939; Lange *et al.*, 1951; Harris *et al.*, 1956) and this leads to the random destruction of a proportion of red cells before the process is reversed by re-oxygenation of the haemoglobin in the lungs. The second change is an increase in the viscosity of the blood (Ham and Castle, 1940; Harris *et al.*, 1956), which, in certain circumstances, leads to stasis of the red cells, with consequent further loss of oxygen and increased sickling. The sickled cells then form thrombi which give rise to the well-known thrombotic symptoms of the disease. These trapped red cells are then lysed, adding further haemolysis to the random destruction of red cells mentioned above. The lessened severity of the different variants of sickle cell disease is well known and appears to be due to the relative resistance to sick-

ling of these cells when compared with sickle cell anaemia cells (Griggs and Harris, 1955). It appears that lower oxygen tensions are necessary for the production of sickling in these cells, as well as for the increase in mechanical fragility and blood viscosity. In sickle cell trait, sickling does not commence until the oxygen tension drops to less than 10 mm. Hg, which is lower than that normally found in the tissues. This finding is, of course, in agreement with the clinical observation that the trait condition only leads to pathology under special circumstances (Levin, 1958). In Hb-SC disease and sickle-cell thalassaemia, the increase in mechanical fragility of the red cells begins at an oxygen tension of 50 mm. Hg. These two diseases are, therefore, almost as severe as sickle cell disease. In sickle-cell persistent Hb-F disease, the increase in mechanical fragility does not begin until the oxygen tension drops to below 30 mm. Hg, and the clinical condition is relatively mild.

In this connexion, it is of interest that *in vitro* sickling of erythrocytes has been reported in association with other abnormal haemoglobins besides Hb-S, namely, Hb- γ_4 (Lie Injo Luan Eng, 1961), Hb-I (Atwater, Schwartz, Erslev, Montgomery, and Tocantins, 1960a) and Hb-C_{Georgetown} (Pierce, Rath, and McCoy, 1963). Murayama (1964) has examined the sickling phenomena in red cells from the subject with Hb-C_{Georgetown} and found that the mechanism differs from that causing sickling in red cells containing Hb-S.

(ii) **Haemoglobin M Disease.** In this group of haemoglobinopathies, the ability to carry oxygen is impaired due to the formation of an unusually stable methaemoglobin derivative. This leads in turn to cyanosis which is present at birth or commences shortly afterwards, but is not associated with the presence of any serious physical disability. The lack of disability distinguishes 'biochemical' methaemoglobinaemia from 'physiological' methaemoglobinaemia (due to congenital heart disease). The biochemical methaemoglobinaemias fall into two groups: those due to deficiency in the methaemoglobin reductase enzyme system (Gibson, 1948; Scott, 1960; Townes and Morrison, 1962) and those due to abnormal haemoglobins; the latter are the Hb-M diseases.

Hörlein and Weber (1948, 1951) were the first to show that primary abnormality in their cases of methaemoglobinaemia lay in the globin part of haemoglobin, while the haem group was normal. The disease is characterized by the appearance of cyanosis at birth or shortly afterwards, without any of the disabilities associated with cyanosis due

FIG. 20. Absorption spectra at pH 7.0 of methaemoglobin A, compared with methaemoglobin M_{Saskatoon} (modified from Gerald and George (1959) with permission).

to congenital heart disease. The prognosis is good. The disease can be distinguished from the other 'biochemical' methaemoglobinaemias due to enzyme abnormalities by definitive differences in the spectrum of the acid methaemoglobin derivative and by the presence of the abnormal haemoglobin. The spectral difference seen is in the region of 550–650 mμ and consists of a shift of the normal absorption maximum 632 to a lower wavelength at approximately 600 mμ (Fig. 20). The degree of these changes varies in the different types of Hb-M described. Minor changes in the spectrum or the presence of Hb-M in whole haemolysates may only lead to filling in of the normal minimum at 600 mμ. The electrophoretic demonstration of the abnormal haemoglobin may be difficult using conventional paper or starch gel electrophoresis, and analysis of the methaemoglobin derivatives at pH 7.0 is recommended (Gerald, 1958).

A large number of patients with Hb-M disease have been described and are reviewed in detail

by Jaffe and Heller (1964). The various haemoglobins M which have been chemically studied are listed in Table II, and it can be seen that several haemoglobins originally described separately are identical.

The stability of the methaemoglobin form and the spectral abnormality can readily be explained on the basis of the amino acid substitution found in these haemoglobins (Gerald and Efron, 1961; Baglioni, 1963a). In two of the Hb-Ms, the substitution is at the histidine residue distal to the haem group which, as has already been noted, is bonded in Hb-A to a water molecule on the sixth co-ordination position of the iron atom. In Hb-M_{Boston}, the histidine in the α -chains is replaced by tyrosine, as is the corresponding histidine (position 63) in the β -chain in Hb-M_{Saskatoon}. The reason why the methaemoglobin form of these two haemoglobins resists reduction to the physiologically useful form of haemoglobin is explained by this substitution, the tyrosine anion being in a position to form a stable complex with the ferric iron of the haem group. Just how critical the location of this residue for the formation of such a complex is, is illustrated by the substitution in the variant Hb-Norfolk. Here, the next amino acid to the distal histidine residue in the α -chain is involved, an aspartic acid residue replacing the glycine (position 51), but no stable methaemoglobin results. This section of the polypeptide chain is an α -helix and therefore the glycine residue points in a direction which is at an angle of approximately 100° to the direction of the histidine residue. The side chain of the fourth residue from the distal histidine, on the other hand, points in roughly the same direction as the histidine, and a substitution here, glutamic acid replacing valine (position $\beta 67$), produces a stable methaemoglobin, namely Hb-M_{Milwaukee 1}.

In Hb-M_{Iwate}, the so-called proximal histidine at position 87 in the α -chain is replaced by a tyrosine residue (Miyaji, Iuchi, Shibata, Takeda, and Tamura, 1963a; Jones, Coleman, and Heller, 1964). In normal haemoglobin, this histidine is linked to the iron of the haem group. Presumably, in Hb-M_{Iwate}, the haem group is held in position by co-ordination to the histidine at position 58 on the opposite side of the haem group, and the new tyrosine residue forms a similar, stable complex with the ferric iron as the tyrosine at position 58 in Hb-M_{Boston}, leading to the usual spectral abnormality.

Another haemoglobin variant that may be related to the haemoglobins M has been reported (Reissmann, Ruth, and Nomura, 1961). This haemo-

globin causes cyanosis due to a lowered oxygen affinity and impaired haem-haem interactions.

Some of the unstable haemoglobins referred to in the next section have their abnormality in the same region of the polypeptide chain, near the haem group. These haemoglobins are associated with haemolytic disease and more rapid methaemoglobin formation in the red cell. Some of them also show differences in the absorption spectrum of the acid methaemoglobin derivative in the region 550–650 m μ , similar to those seen in the Hb-Ms. Conversely, it might be expected that some of the M-type haemoglobins would show some instability with consequent haemolytic disease. A shortened ^{51}Cr -labelled red cell survival and increase in reticulocytes have been reported in association with Hb-M_{Chicago} (Saskatoon) (Josephson *et al.*, 1962). In several other instances a raised reticulocyte count has been reported (Heck and Wolf, 1958) (3.3%), Hb-M_{Milwaukee 1} (2.3%) (Pisciotta, Ebbe, and Hinz, 1959), Hb-M_{Milwaukee 2} (4%) (Pisciotta *et al.*, 1959). There was definitive haemolysis in the patient who carried both Hb-C trait and Hb-M_{Milwaukee 2}, whereas in Hb-C trait the reticulocyte count was normal. It thus appears that some haemolysis is present in a number of patients with Hb-M disease.

(iii) The 'Unstable Haemoglobin' Diseases.

A number of abnormal haemoglobins has recently been discovered in which an amino acid substitution produces a molecule that is less stable than the normal form, and a number of haemoglobinopathies due to such haemoglobins have now been described.

HAEMOGLOBIN ZÜRICH DISEASE. This is a familial haemoglobinopathy (Frick, Hitzig, and Betke, 1962; Bachmann and Marti, 1962) characterized by severe haemolytic crises precipitated by oral sulphonamides. During the crises, the haemoglobin drops to 3–6 g. per 100 ml. with a high (40%) reticulocytosis. The red cells in the stained smear show marked anisocytosis and fragmentation, with the presence of many large inclusion bodies thought to be precipitated haemoglobin. During the quiescent stage of the disease there is very little anaemia, but haemolysis is still present, as shown by a raised reticulocyte count and red cell survival.

The abnormal haemoglobin is easily detected by electrophoresis at pH 8.6, migrating on paper or starch gel with Hb-S. The amino acid substitution is in the β -chains, the so-called distal histidine at position 63 being replaced by arginine

(Muller and Kingma, 1961). The rate of methaemoglobin formation in the red cells incubated *in vitro* at 37°C. is significantly increased when compared to normal controls. No spectral abnormalities of this haemoglobin have been reported.

HAEMOGLOBIN UBE-1 DISEASE. This disease has been reported in one person from Japan (Shibata, Iuchi, Miyaji, Ueda, and Takeda, 1963). His parents did not have any abnormality. It is a severe haemolytic disorder associated with Heinz body formation. The condition of the patient was considerably improved by splenectomy.

The abnormal haemoglobin is easily separated by electrophoresis, amounting to approximately 10% of total haemoglobin. It migrates between Hb-S and Hb-C. Structural studies indicate that this abnormality is in the insoluble tryptic peptides (X-XII) of the β -chain with loss of reactivity of the sulphhydryl at position 92.

HAEMOGLOBIN SEATTLE DISEASE. This disease is a mild non-spherocytic haemolytic anaemia with a haemoglobin of approximately 11 g. % and 2-4% reticulocytes (Huehns, E. R., Hartmann, J., Hecht, F., and Motulsky, A. G., unpublished). In the affected family, three heterozygous carriers of the abnormal haemoglobin in two generations have the disease, making it likely that the haemoglobin is directly related to the disease.

The haemoglobin is distinctive in its electrophoretic properties. It does not separate from Hb-A by paper or starch block electrophoresis in the usual barbiturate buffers at pH 8.6, though the amount of Hb-A₂ appears somewhat increased. It is also not separable on starch gel electrophoresis in the *tris*-citrate/borate discontinuous system of Poulik (1957). Thus, this abnormal haemoglobin cannot be detected by the usual electrophoretic systems. Hb-Seattle can, however, be easily separated by starch gel electrophoresis, using a sodium phosphate buffer at pH 7.4 or a *tris*-E.D.T.A./borate buffer at pH 8.6. It amounts to 40% of total haemoglobin in the red cells. The abnormal haemoglobin is less stable than Hb-A to heat denaturation.

Though there are no inclusion bodies or methaemoglobin in fresh red cells, both appear more rapidly than in normal cells on *in vitro* incubation. The absorption spectrum of the acid methaemoglobin derivative of Hb-Seattle is slightly abnormal in the region of 550-650 m μ , showing some filling in of the normal minimum at 600 m μ . Structural studies indicate that the abnormality is in the β -chains, an alanine residue being replaced

by glutamic acid at position 70 or 76 in peptide β TpIX.

HAEMOGLOBIN ST. MARY'S DISEASE. Hb-St. Mary's disease (Buchanan, A., Barkhan, P., Crome, P. E., Mollison, P. L., and Huehns, E. R., unpublished) is a well-compensated haemolytic disease with a shortened red cell life-span and 10% reticulocytes. The patient had a number of haemolytic crises with moderately severe anaemia. The disease is distinguished from the other haemolytic anaemias due to abnormal haemoglobins in that the abnormal haemoglobin cannot be resolved by paper or starch block electrophoresis in barbiturate buffer, pH 8.6, or by starch gel electrophoresis in *tris*-citrate/borate buffer, pH 8.6, or phosphate buffer, pH 7.4. However, Hb-St. Mary's can be separated by starch gel electrophoresis in a *tris*-E.D.T.A./borate buffer, pH 8.6, when it migrates close to Hb-S. It amounts to 11% of total haemoglobin. Structural studies of the isolated haemoglobin indicate that the abnormality is probably in the insoluble (core) tryptic peptides of the β -chains. The possibility that the abnormality is close to the haem group is suggested by the finding that the absorption spectrum of the acid methaemoglobin derivative is abnormal in the region 550-650 m μ . The haemoglobin is unstable and is more readily denatured by heat than is normal haemoglobin (Fig. 21).

Both Hb-Seattle and Hb-St. Mary's are haemoglobins which are more unstable *in vitro* than Hb-A, and this instability can be shown in various ways, one of which is heat denaturation. However, studies by Sephadex chromatography show that the instability of the molecule leads to both increased dissociation of the molecule as well as polymer formation at neutral pH*. This tendency is shown to a significantly greater degree by Hb-St. Mary's than Hb-Seattle, which is consistent with the *in vivo* pathological effect of the two haemoglobins. It is also of interest that Hb- α^A is found in the haemolysate containing these haemoglobins, indicating that the β -chains—the abnormal chains—of these haemoglobins are more unstable than the complementary normal α -chains.

HAEMOGLOBIN KÖLN DISEASE. Pribilla (1962) has described a family in which some members have a mild haemolytic disease associated with an abnormal haemoglobin and the formation of

* *In vitro* polymer formation has been noted in one human haemoglobin without any associated disease in the heterozygous or homozygous states (Tondo, Salzano, and Rucknagel, 1963).

FIG. 21. Starch gel electrophoresis *tris*-E.D.T.A.-borate buffer pH 8.6 of haemolysate containing Hb-A, Hb-A₂, and the unstable haemoglobin Hb-St. Mary's: (i) after incubation at 70° C. for 1 minute, note absence of unstable haemoglobin; (ii) untreated control.

Heinz bodies. The abnormal haemoglobin amounted to 5% of total haemoglobin and on electrophoresis it migrated close to Hb-S. More recently, a second family, with the same abnormal haemoglobin, has been described in Scotland (Hutchison, Pinkerton, Waters, Douglas, Lehmann, and Beale, 1964). In this family, the haemoglobin abnormality was found in three generations, and each affected person showed haemolytic anaemia with Heinz body formation. There was some improvement of the disease on splenectomy. Structural studies show that the haemoglobin has abnormal β -chains with the abnormality in the insoluble (core) tryptic peptides. As with Hb-Seattle and Hb-St. Mary's, free α^A -chain haemoglobin was found in the haemolysate (Huehns, E. R., unpublished). The electrophoretic properties of Hb-Köln distinguish it from Hb-St. Mary's and Hb-Seattle.

THE 'HEAT-UNSTABLE HAEMOGLOBIN' DISEASES. Two families with this type of unstable haemoglobin have been described (Grimes and Meisler, 1962; Grimes, Meisler, and Dacie, 1964; Dacie, Grimes, Meisler, Steingold, Hemsted, Beaven, and White, 1964). The first patient (Grimes *et al.*, 1964) had a severe haemolytic anaemia with a haemoglobin of 6.8 g. per 100 ml., and 22–47% reticulocytes. Following splenectomy there was some clinical improvement, and the red cells were found to contain many Heinz bodies. No

abnormal haemoglobin could be detected by electrophoresis on paper or starch gel of fresh haemolysates. However, a proportion (approximately 35%) of the haemoglobin was relatively unstable and denatured readily on heating to 50° C. Both parents of this child are alive and well and have normal blood counts.

In the second family (Dacie *et al.*, 1964) a mild haemolytic anaemia was found in four generations of the same family. The haemoglobin values ranged from 10–14 g. per 100 ml. with reticulocyte counts of 10%. Heinz bodies were found only in the red cells of one patient who had previously been splenectomized. However, Heinz bodies could be detected in the red cells of the other affected individuals after *in vitro* incubation of the red cells.

On heating the haemoglobin solution to 50° C. for one hour, a precipitate of haemoglobin developed, indicating the presence of some heat-unstable haemoglobin. On paper electrophoresis, only some trailing pigment behind Hb-A was seen. On starch gel electrophoresis in a *tris*-citrate/borate buffer system at pH 8.6, three abnormal zones could be seen besides Hb-A, Hb-A₂, and Hb-A₃. Two of these migrated between Hb-A and Hb-A₂, and one just slower than Hb-A₂. The total amount of abnormal pigment was approximately 15%. The three abnormal zones seen on starch gel electrophoresis may represent aggregates of haemoglobin with different molecular weights being

retarded to differing extents by the pores in the gel. At pH 6.9, in phosphate buffer, a small zone migrating more rapidly toward the cathode was seen. This extra zone was not seen after the addition of cyanide. Further study shows that this largely consists of free haem (G. H. Beaven, personal communication), and this finding may indicate that the haem-protein linkage in this haemoglobin is less stable than in normal haemoglobin.

There are a number of other reports of haemolytic anaemias in association with inclusion bodies (Lange and Akeroyd, 1958; Schmid, Brecher, and Clemens, 1959; Scott, Haut, Cartwright, and Wintrobe, 1960), and it appears likely that at least some of these will eventually be shown to be associated with an abnormal haemoglobin.

From the diseases described in this section, it is clear that a proportion of the non-spherocytic haemolytic anaemias is due to the presence of abnormal haemoglobins in the heterozygous state and that the haemolytic process is directly related to the instability of the haemoglobin. This direct relation between the presence of an abnormal unstable haemoglobin and disease seems certain in those studies where several members of a family in more than one generation are affected. In other reports, where only an isolated case has been studied, the relation is not at all clear. In these instances, the abnormal haemoglobin may be a chance finding or, alternatively, only part of the story, the disease only being manifest when the abnormal haemoglobin is accompanied by some unidentified interacting factor. A detailed study of other red cell components is clearly indicated in all cases. The difficulty of detecting some of the haemoglobin abnormalities suggests that other cases of unexplained haemolytic anaemia may also be due to the presence of an abnormal haemoglobin and that this may be detected only by the most careful studies. The properties of the various unstable haemoglobins described suggest that, in screening patients for this type of haemoglobin abnormality, electrophoresis of both the oxy- and the methaemoglobin derivatives in starch gel, using a number of different buffer systems at several pH values, should be carried out. The finding of Hb- α^A in the haemolysate or the presence of a heat-unstable fraction may be the first indication that one is dealing with a haemoglobin abnormality.

The reasons why these haemoglobins are less stable than Hb-A are not clear. It can be postulated that the presence of a different amino acid in the critical region near the haem group causes a

strain in the molecule leading to some conformational changes, thus producing a less stable molecule.

(iv) **Haemoglobinopathies Associated with the Homozygous State.** These have been well described and comprise Hb-C disease (Spaet, Alway, and Ward, 1953), Hb-E disease (Lehmann, Story, and Thein, 1956; Na-Nakorn and Minnich, 1957), and Hb-D disease (Bird and Lehmann, 1956).

There is mild haemolysis which is not fully compensated, leading to haemoglobin levels of 10–12 g. per 100 ml. The stained blood film shows some hypochromia and target cells. These diseases are found in subjects who are homozygous for any one of the above abnormal haemoglobins, the heterozygous condition being benign. The cause of the haemolysis in these syndromes is not clear but may arise because these haemoglobins show a slight instability which is not enough to cause disease when present together with Hb-A. It is also puzzling that in these cases the mild haemolysis is not fully compensated by increased red cell production, though more severe haemolysis, as, for example, in Hb-St. Mary's disease, shows so much better compensation.

The Thalassaemias

The characteristic abnormality in thalassaemia is the reduction in the amount of haemoglobin in the red cells due to diminished haemoglobin synthesis. As haemoglobin consists of the prosthetic haem group joined to the protein moiety, globin, impairment of haemoglobin synthesis could be due either to diminished synthesis of haem or globin.

The finding of Bannerman, Grinstein, and Moore (1959) of impaired incorporation of ^{14}C -glycine into haem and some haem precursors in *in vitro* bone marrow cultures prepared from patients with thalassaemia appeared to support the idea that the prime abnormality of this disease was an abnormality of haem synthesis. On the other hand, the well-documented clinical observation that infants with thalassaemia major are normal at birth, developing the disease at about 3–6 months of age, is opposed to the idea that the primary abnormality is a defect in haem synthesis. This is because the haem groups of foetal haemoglobin and adult haemoglobin are identical, namely, ferrous protoporphyrin IX, and hence impairment of its synthesis should lead to anaemia during foetal as well as adult life. On the other hand, the protein part of these molecules differs, containing

γ -chains in foetal haemoglobin ($\alpha_2\gamma_2$) and β -chains in adult haemoglobin ($\alpha_2\beta_2$). Recent work has shown that haem exerts a negative feedback on the synthesis of δ -amino-laevulinic acid (ALA) in red cell precursors (Lascelles, 1960; Burnham and Lascelles, 1963; London, 1964; Steiner, Baldini, and Dameshek, 1964). The reduced rate of haem synthesis in thalassaemia can thus be explained by 'feedback' inhibition of the synthesis of δ -ALA by the un-utilized haem formed in the thalassaemic red cell. Finally, Marks and Burka (1964) have shown that the synthesis of β -chains is impaired in the 'cell-free system' prepared with ribosomes from β -thalassaemic red cell precursors. From these considerations it appears that thalassaemia is an abnormality of globin synthesis.

It has already been noted that the α - and β -chains of haemoglobin are synthesized independently and are under separate genetic control. This implies that, just as there are two main groups of abnormal haemoglobins, α -chain variants and β -chain variants, there would also be two groups of thalassaemias, one affecting the synthesis of α -chains and one affecting the synthesis of β -chains, and these have been called α -thalassaemia and β -thalassaemia respectively (Ingram and Stretton, 1959). As there are also δ - and γ -chain haemoglobin variants, it might be expected that δ - and γ -thalassaemia also occur. One person without any Hb-A₂ has been reported, and he may be an example of δ -thalassaemia (Fessas and Stamatoyannopoulos, 1962). The existence of γ -thalassaemia has also been claimed (Hamilton, Sheets, and Brosseau, 1962). However, the only two established groups are α - and β -thalassaemia.

The genes causing thalassaemia can, of course, occur in the heterozygous or homozygous state. Furthermore, it might be envisaged that there are a number of different mutations leading to varying degrees of impairment of polypeptide chain synthesis. Some mutations would completely, or almost completely, inhibit the synthesis of a particular polypeptide chain, while others would only partially inhibit it. This hypothesis implies that there is a whole range of mutations causing varying degrees of inhibition in the synthesis of either the α - or β -chains. In the simple heterozygote it is difficult to measure the degree of inhibition of any particular thalassaemia gene. However, an approximate measure of the degree of inhibition caused by a given thalassaemia gene is shown by the proportion of Hb-A found in subjects who carry an abnormal haemoglobin. This is illustrated in mixed heterozygotes for β -thalassaemia and Hb-S or Hb-C. If no Hb-A

is found, it may be inferred that complete suppression of β -chains has been caused by the thalassaemia gene. In others, various amounts of Hb-A are found, due to different degrees of inhibition of β -chain synthesis. The same argument applies to α -thalassaemia interacting with haemoglobins with abnormal α -chains. A full review of the thalassaemias has recently been published by Motulsky (1964b).

Classification. On the basis of the above hypothesis, the various thalassaemic syndromes that have been described can be classified as shown below. It should be pointed out that though this classification does not embrace every described variant of thalassaemia, it does provide a useful way of categorizing these syndromes.

(i) β -THALASSAEMIA

(a) *β -thalassaemia Trait.* This is a mild anaemia with the morphological changes in the blood smear typical of thalassaemia as well as a raised Hb-A₂ (Gerald and Diamond, 1958a). The increase in the proportion of Hb-A₂ is thought to be due to the continued synthesis of Hb-A₂ at a normal rate while that of Hb-A is impaired. In a proportion of these subjects, the foetal haemoglobin is also raised 2-5% above the normal maximum level of 0.4%. In those who carry a β -thalassaemia gene, which only partially inhibits the synthesis of Hb-A, the proportion of Hb-A₂ might be only slightly increased. Several authors (Zuelzer, Robinson, and Booker, 1961; Malamos, Fessas, and Stamatoyannopoulos, 1962; Fessas, 1964) have described a form of thalassaemia trait affecting the synthesis of β -chains in which Hb-A₂ is present in normal concentration but Hb-F is increased, and it is possible that this is due to a mutation suppressing both β - and δ -chain synthesis (β - δ -thalassaemia) (Motulsky, 1964b).

(b) *β -thalassaemia Major.* This is the classical thalassaemia major. Family studies have shown that these persons have inherited two β -thalassaemia genes (Gerald and Diamond, 1958b). Because β -chain synthesis is almost completely inhibited, a severe disease results which begins at the time in development when γ -chain synthesis normally ceases and β -chain synthesis commences. The severe anaemia produces a stress situation in the erythroid marrow, which, by a mechanism not yet understood, leads to the continuation of Hb-F synthesis but at a rate far below that necessary for complete compensation. It appears that the Hb-F produced by such a situation is unevenly distributed among the red cells (cf., sickle cell

disease—Singer and Fisher, 1952). This results in the production of red cells containing varying amounts of haemoglobin. Those cells that contain very little haemoglobin are destroyed in the marrow, the so-called 'ineffective erythropoiesis' which is a prominent feature of the disease. The remaining (smaller) proportion reaches the circulation.

One of the puzzling features of β -thalassaemia is that though the proportion of Hb-A₂ is raised in β -thalassaemia trait, it is normal or low in the homozygous state. If δ -chain synthesis were proceeding at a normal rate, it might be expected that Hb-A₂ would be much higher in the homozygous condition than in the trait form. A possible explanation is that the continued synthesis of γ -chains suppresses that of the δ -chains by some kind of 'feedback' mechanism.

(c) *β -thalassaemia Intermedia*. As the name implies, this disease is of intermediate severity to β -thalassaemia major and minor. It is characterized by a haemoglobin of 6–10 g. per 100 ml. with the typical blood picture of thalassaemia. Usually, there is gross splenic enlargement as well as the bone changes seen in thalassaemia major. Hb-F is raised from 20–80% and the proportion of Hb-A₂ is often greater than normal. Family studies often do not show the β -thalassaemia trait in both parents, and it is presumed that genetically this condition is due to the interaction of two β -thalassaemia genes, one of which completely inhibits β -chain synthesis, while the other causes only partial inhibition. This clinical syndrome is also found in patients suffering from Hb-Lepore-thalassaemia disease (Gerald and Diamond, 1958b; Fessas *et al.*, 1962; Neeb *et al.*, 1961; Pearson, Gerald, and Diamond, 1959; Beaven, Gratzer, Stevens, Shooter, Ellis, White, and Gillespie, 1964) or homozygous Lepore disease (Neeb *et al.*, 1961; Fessas *et al.*, 1962). In both these conditions, a large proportion of the haemoglobin formed in the red cells is Hb-F. Milder clinical syndromes of this type are the well-documented interactions of abnormal haemoglobins such as Hb-C, Hb-E, or Hb-D with β -thalassaemia described below.

(d) *Interaction of β -thalassaemia with a β -chain Variant of Hb-A*. Hb-S thalassaemia disease is a clinical variant of sickle cell disease (Singer, Singer, and Goldberg, 1955). Hb-C-thalassaemia disease (Singer, Kraus, Singer, Rubinstein, and Goldberg, 1954; Zuelzer and Kaplan, 1954), Hb-D-thalassaemia disease (Hynes and Lehmann, 1956), and Hb-E-thalassaemia disease (Na-Nakorn and Minnich, 1957; Kochhar and Kathpalia, 1963) are some interactions of this type. Clinically,

these are variants of thalassaemia of intermediate to mild severity. The diagnosis is made from the clinical picture and the electrophoretic analysis of the haemolysates. Often the abnormal haemoglobin and Hb-A will be found, but the proportion of Hb-A is lower than 50%, whereas in the simple abnormal haemoglobin trait Hb-A usually exceeds the abnormal haemoglobins in amount. In some patients, no Hb-A is synthesized, and the differentiation from the homozygous haemoglobinopathies rests on family studies. Hb-Lepore-thalassaemia disease (Gerald and Diamond, 1958b) is a much more severe disease closely resembling thalassaemia major, with a haemoglobin level of approximately 8 g. per 100 ml. The main pigment in the red cells is Hb-F (65–70%) with 5–7% Hb-Lepore and a low level of Hb-A₂.

(ii) *INTERACTION OF α - WITH β -THALASSAEMIA* (Fessas, 1962). Clinically, this disease is a form of thalassaemia minor. The diagnosis can only be established by family studies.

(iii) *α -THALASSAEMIA*. As the α -chains of Hb-A, Hb-F, and Hb-A₂ are identical, α -thalassaemia affects haemoglobin production both before birth as well as later in life, and it appears that the effect is clinically more severe during foetal life than later.

(a) *α -thalassaemia Trait*. This trait is somewhat milder than the β -thalassaemia trait, presumably because there is good compensation by the remaining normal Hb α gene. The clinical features of the trait in persons in whom one Hb α gene is completely inhibited have been outlined by Malamos *et al.* (1962). At birth, these people carry 5–10% Hb- γ_4 , and this disappears during the neonatal period together with Hb-F. Later in life, the patient has a slight anaemia, with a blood film suggesting thalassaemia. The Hb-A₂ level is normal. There are no clinical signs. The diagnosis can be confirmed by finding occasional cells with inclusion bodies after incubation with brilliant cresyl blue. In a few patients, a trace of Hb- γ_4 is found in the haemolysate.

Subjects who inherit an α -thalassaemia gene which only partially suppresses the synthesis of α -chains have no detectable abnormality later in life but at birth have a slight increase of Hb- γ_4 in the range of 1–2%.

(b) *α -thalassaemia Major*. This is due to the inheritance of two α -thalassaemia genes causing severe α -chain suppression (see above). In these subjects, no α -chains are synthesized, and death *in utero* apparently occurs at approximately 32

weeks' gestation and the foetus shows severe hydrops foetalis, anaemia, and erythroblastosis. (Lie-Injo Luan Eng and Jo Biwan Hie, 1960; Lie-Injo Luan Eng and Lie Hong Gie, 1961; Lie-Injo Luan Eng, 1962).

(c) *α -thalassaemia Intermedia or Haemoglobin H Disease.* Recent work on Hb-H disease leaves little doubt that it is caused solely by a deficiency of α -chain synthesis. Since the α -chains for the three normal haemoglobins, Hb-A, Hb-A₂, and Hb-F, are derived from a common metabolic pool, α -chain deficiency should lead not only to the formation of Hb- β_4 , but also in the same person to the formation of haemoglobins consisting solely of γ - and δ -chains, Hb- γ_4 and Hb- δ , respectively. The fact that this occurs has recently been demonstrated in four patients with Hb-H disease (Dance, Huehns, and Beaven, 1963). The finding that some of the δ -chains synthesized are used in the formation of Hb- δ accounts for the low levels of Hb-A₂ found consistently in the disease. Secondly, the occurrence of Hb- γ_4 without Hb- β_4 in some of the individuals who are synthesizing both β - and γ -chains must be explained. It has previously been suggested that the available α -chains are used preferentially for the formation of Hb-A rather than Hb-F (Huehns, Flynn, Butler and Shooter, 1960), and this has been confirmed by *in vitro* recombination experiments using the isolated chain subunits (Huehns, Beaven, and Stevens, 1964e). When the published pedigrees of families with Hb-H disease are studied, it appears likely that the disease is caused by the interaction of two abnormal genes. The occurrence of detectable α -thalassaemia trait in some of the relatives is well documented. Huehns *et al.* (1960) have suggested that Hb-H disease is due to two α -thalassaemia genes. The work of Lie Injo Luan Eng already referred to indicates that the inheritance of two severe α -thalassaemia genes leads to intrauterine death. It is therefore suggested that Hb-H disease is caused by the interaction of a severe α -thalassaemia gene (causing complete suppression of α -chain synthesis) with a milder α -thalassaemia gene (causing partial suppression of α -chain synthesis) (Huehns, 1962). As the incidence of the severe type of α -thalassaemia genes is known (Malamos *et al.*, 1962), the occurrence of families in which Hb-H appears in several generations (Gouttas *et al.*, 1955; Bingle, Huehns, and Prankerd, 1958; Ramot, Sheba, Fisher, Ager, and Lehmann, 1959; Minnich, Na-Nakorn, Tuchinda, Pravitt, and Moore, 1956) indicates that the mild α -thalassaemia gene is relatively common, occurring in approximately 20% of the population.

The published family data of Hb-H disease are consistent with this hypothesis (E. R. Huehns and A. G. Motulsky, unpublished; Wasi, Na-Nakorn, and Suingdamrong, 1964). Confirmation of this explanation for Hb-H disease could be obtained if it is shown that all newborns from parents with the disease carry some Hb- γ_4 , and also if the incidence in the affected population of the mild type of α -thalassaemia gene postulated above could be shown to be approximately 20%. To date five newborns from patients with Hb-H disease have been examined and four of these carried increased amounts of Hb- γ_4 (Fessas 1960; E. R. Huehns, unpublished). It is, however, not known whether the methods used would have detected the small increases postulated to occur in some of these cases.

(d) *The Interaction of α -thalassaemia With an α -chain Variant of Haemoglobin A.* Hb-Q- α -thalassaemia disease clinically resembles Hb-H disease, except that instead of Hb-A, Hb-Q is found (Vella, Wells, Ager, and Lehmann, 1958; Dormandy *et al.*, 1961; Lie-Injo Luan Eng and Hart, 1963). Because no Hb-A is present in these cases, the α -thalassaemia would be of the severe type. One patient with Hb-I- α -thalassaemia disease has been described (Atwater *et al.*, 1960a). Clinically, the picture was that of thalassaemia. The patient's haemolysate showed approximately 80% Hb-I and 20% Hb-A; Hb-A₂ and Hb-I₂ were also present (Atwater *et al.*, 1961). In this case, the α -thalassaemia allowed the formation of significant amounts of Hb-A and was therefore of the mild type.

The Genetic Abnormality in Thalassaemia. During the past few years, consideration has been given to the type of mutation that gives rise to thalassaemia. Studies of offspring in families in which β -thalassaemia and abnormal haemoglobins occur indicate that the genetic loci controlling the synthesis of the β -chains and those causing β -thalassaemia are either allelic or closely linked (see above). Several hypotheses have been advanced.

(a) The rate of haemoglobin synthesis is diminished because the 'Hb-A' synthesized in these persons is not normal Hb-A but contains a 'silent' amino acid substitution (Itano, 1957; Ingram and Stretton, 1959). The finding (Guidotti, G., quoted by Baglioni, 1963a) that the amino acid composition of isolated peptides from two such samples of 'Hb-A' was identical to normal Hb-A argues against this idea but does not altogether exclude it.

(b) The alternative view that the reduced rate of synthesis is due to a mutation of a closely-linked rate-controlling locus, such as the operator locus postulated in the control of bacterial protein synthesis by Jacob and Monod (1961), has been discussed by a number of workers (Neel, 1961; Motulsky, 1962; Sturgeon *et al.*, 1963; Zuckerkandl, 1964). However, these hypotheses do not explain all the findings in thalassaemia.

(c) Recently, Itano (1964) has advanced a third hypothesis. This depends on the idea that the genetic code is degenerate, that is, that two different triplet code words (see above) can code for the same amino acid. In normal persons, the normal triplet code word is present, but in thalassaemic persons, the normal code triplet is replaced by a different one which codes for the same amino acid. One of the factors that limits the rate of addition of any particular amino acid to the growing end of a polypeptide chain during synthesis is the availability of amino acid transfer-RNA fitting any particular coding triplet, and it is argued that there is plenty of transfer-RNA to fit the normal coding triplet but a deficiency of transfer-RNA for the abnormal triplet. This deficiency of availability of a particular amino acid leads to a block in the synthesis of the affected polypeptide chain and the decreased normal globin production seen in thalassaemia.

(d) Ingram (1964) has put forward still another hypothesis. This proposes that in thalassaemia the messenger RNA for the affected chain is abnormal and blocks some of the ribosomal sites of protein synthesis so that they are not available for haemoglobin synthesis.

(e) Another possibility is that the messenger RNA formed, if abnormal, would be less stable than normal in mRNA. Each mRNA would then only be available for the synthesis of a smaller number of polypeptide chains than its normal counterpart.

The multiplicity of hypotheses put forward to explain the findings in thalassaemia indicates that at the present time the data on which a sound theory can be based are not yet available, and it is possible that there is more than one cause of thalassaemia.

The Hereditary Persistence of Foetal Haemoglobin

A number of investigators have described a benign condition in Negroes in which the production of Hb-F continues into adult life, 20–30% of total haemoglobin being foetal in type, the re-

mainder being Hb-A and Hb-A₂ (Edington and Lehmann, 1955a, b; Jacob and Raper, 1958; Went and MacIver, 1958; Herman and Conley, 1960; Bradley *et al.*, 1961; Kraus, Koch, and Burkett, 1961; Thompson, Mitchener, and Huisman, 1961; Conley *et al.*, 1963). It occurs in about 1% of American Negroes. The distribution in Africa is not known in detail, but about 1% of the population is affected in West Africa (Conley *et al.*, 1963). Several studies have indicated that the foetal haemoglobin in these persons is the same as that found in normal cord blood (Thompson *et al.*, 1961; Schroeder, Sturgeon, and Bergren, 1962; Baglioni, 1963b). The foetal haemoglobin is uniformly distributed in the red cells (Thompson *et al.*, 1961; Bradley *et al.*, 1961). This contrasts with the finding of uneven Hb-F distribution in the red cells in other conditions, such as sickle cell anaemia (Singer and Fisher, 1952) or thalassaemia major (Shepard, Weatherall, and Conley, 1962), where the persistence of Hb-F is not the direct result of the genetic abnormality but secondary to a stress situation in the bone marrow.

When a person inherits this condition as well as a gene causing the production of a haemoglobin variant with abnormal β -chains, no Hb-A is produced*. When such persons are mated to normal persons, their children carry either the 'high F gene' or the abnormal haemoglobin (Thompson and Lehmann, 1962; Conley *et al.*, 1963; see also Rucknagel and Neel, 1961), indicating that the 'high F gene' is closely linked or allelic to the Hb β locus. Whether the γ -chain structural locus is linked to that at which the 'high F gene' is situated is not known. Data on this point will come from the study of families in which both the 'high F gene' and a γ -chain variant of Hb-F are found.

Besides the interaction with abnormal haemoglobins, interaction with β -thalassaemia has been reported. These patients have approximately 65–70% Hb-F and the clinical picture of thalassaemia intermedia (Kraus *et al.*, 1961; Wheeler and Krevans, 1961; Barkham and Adinolfi, 1962).

One person probably homozygous for the 'high F gene' has been reported (Wheeler and Krevans, 1961). He had only slight haematological abnormality; he carried only Hb-F, and no Hb-A or Hb-A₂ could be detected.

* These people carry these traits in repulsion. If the 'high F gene' occurred in coupling to an abnormal Hb β gene, the production of the corresponding abnormal haemoglobin would be suppressed and the phenotype could not be distinguished from the simple trait. The same applies to the progeny of the matings mentioned, and the only cross-over that could be distinguished would be one that leads to a normal person.

Several hypotheses to explain these findings have been advanced. Wheeler and Krevans (1961) suggest that it might be due to a deletion of the Hb β and Hb δ loci. The deletion must also lead to the continued production of Hb-F in sufficient amounts to compensate for the lack of Hb-A production. Other workers (Neel, 1961; Motulsky, 1962; Sturgeon *et al.*, 1963; Zuckerkandl, 1964) have postulated that it is due to a mutation affecting the regulatory mechanism controlling the Hb β and Hb δ loci, leading to failure to turn on the synthesis of these chains and at the same time failure to turn off γ -chain production.

Two further types of inherited persistence of foetal haemoglobin have been described, which differ from the 'high F gene' described above in that the amount of Hb-F found in the heterozygotes is significantly lower. The first occurs in 0.25% of Greeks (Fessas and Stamatoyannopoulos, 1964). In these individuals, Hb-F levels vary from 11–18%, and Hb-A $_2$ is within the lower range of normal. There are no haematological abnormalities, and Hb-F is evenly distributed in all red cells. This trait has also been described in association with β -thalassaemia; clinically, this is a form of β -thalassaemia intermedia, with 20–40% Hb-F and a raised Hb-A $_2$. The other type occurs in about 1% of the population of Southern Switzerland. It is not associated with any clinical or haematological abnormality (Marti and Bütler, 1961; Marti, 1963).

The Occurrence of Haemoglobin Abnormalities in Other Diseases

Haemoglobin A $_2$. Variation between normal people in the level of Hb-A $_2$ is small, and there is also little variation in disease besides that occurring in thalassaemia. There is one report that Hb-A $_2$ is raised in pernicious anaemia and low in iron-deficiency anaemia (Josephson, Masri, Singer, Dworkin, and Singer, 1958). Another report suggests that Hb-A $_2$ is raised after bone marrow transplantation (Bridges, Neill, and Lehmann, 1961). From the small amount of data available, it is not possible to determine whether the above changes are present in all patients.

Haemoglobin F (for review, see Beaven *et al.*, 1960b). Delayed disappearance of Hb-F after birth is associated with a number of conditions such as prematurity (Jonxis, 1951), congenital spherocytosis, and other congenital haemolytic anaemias. The foetal haemoglobin levels in these infants eventually reach normal levels. There is

also a delay in the disappearance of Hb-F in trisomy 13–15 and in a proportion of Negroes with Down's syndrome (trisomy 21–22) already referred to.

In acute leukaemia in young children, high Hb-F levels are often found. In adult patients with leukaemia, small increases in Hb-F are occasionally found, particularly in patients with erythroleukaemia. A raised Hb-F has also been reported in aplastic anaemia (Jones, 1961; Jonxis, 1961). In pernicious anaemia, Hb-F is present in small amounts (1–2%) in approximately half the patients examined. It is of interest that the proportion of this pigment often rises shortly after the commencement of treatment with vitamin B $_{12}$ before the maximum reticulocyte response has been reached (Beaven *et al.*, 1960b).

Small amounts of foetal haemoglobin have also been found in approximately 10% of women during the second trimester of pregnancy by a sensitive immunological technique. These slightly raised levels fall to normal adult levels in the last trimester (Rucknagel and Chernoff, 1955). The occasional finding of high Hb-F levels in late pregnancy may indicate transplacental foetal haemorrhage. Bromberg, Salzberger, and Abrahamov (1957) report a significantly raised Hb-F in four patients with a molar pregnancy.

Iron deficiency, acquired haemolytic anaemias, and congenital cyanotic heart disease are not associated with a raised Hb-F.

Haemoglobin H. A number of patients with Hb-H in association with erythroleukaemia have been described (Bergren and Sturgeon, 1960; White, Ellis, Coleman, Beaven, Gratzner, Shooter, and Skinner, 1960; Beaven, Stevens, Dance, and White, 1963; C. A. Finch, A. G. Motulsky, unpublished). The red cells in these patients show hypochromia and some target cells and resemble those seen in the congenital form of Hb-H disease. As these patients come from populations in which Hb-H disease has not been reported, it appears that the appearance of this abnormal haemoglobin is due to the leukaemia. In two of them, small amounts of Hb- γ_4 were also present (E. R. Huehns, unpublished). Another report of an unstable haemoglobin associated with leukaemia comes from India (Mathur and Misra, 1962).

Sickle Cell Trait. There is one report of a variation in the proportion of Hb-A to Hb-S in megaloblastic anaemia (Heller, Yakulis, Epstein, and Friedland, 1963). It was found that the proportion of Hb-S present was unusually low when the

patient was first seen. After recovery from the anaemia, the proportion of Hb-S increased significantly.

Animal Haemoglobins. Van Vliet and Huismans (1964) have studied experimental anaemia in sheep and shown definitive changes in the relative proportion of types of haemoglobin present in the red cells. If the experimental animals were allowed to recover, the haemoglobin pattern returned to that at the beginning of the study.

Distribution of Abnormalities of Haemoglobin Synthesis

Since the discovery of the abnormal haemoglobin, it has been shown that some, such as Hb-S, Hb-C, Hb-D, and Hb-E, occur quite frequently in some parts of the world, while in others they are only found occasionally. It has also been found that the thalassaemias only have a high incidence in certain areas. The harmful, and often lethal, effects of these inherited abnormalities in the homozygous state raise the problem of why these deleterious genes are not rapidly eliminated from the population. Theoretically, this can be prevented if the heterozygous state confers some compensating advantage (heterosis) and is fitter in the Darwinian sense than either the normal or abnormal homozygote. At certain frequencies the harmful effects of the abnormal gene in a given population will be exactly balanced by the conferred advantage. Such an equilibrium is stable and is known as a balanced polymorphism (Stern, 1960). In other words, if homozygotes die prematurely or are so infertile that they seldom reproduce (and hence do not transmit the abnormal gene), their Darwinian fitness is said to be near zero. The gene can, nevertheless, be maintained in the population if the heterozygotes are fitter than the normal homozygotes in this sense. This increased fitness may be due to a relative immunity to some disease which can cause premature mortality or impairs fertility. Clearly, such a disease must be prevalent in the areas in which the relevant red cell traits are found and much effort has been directed towards identifying such factors. Haldane (1949) suggested that thalassaemia protected against malaria. Allison (1954) drew attention to the possible role of sickle cell trait in protecting against malignant tertian malaria and provided the first data in support of this hypothesis. He also pointed out that in a hyperendemic region the effect should be most marked during the first years of life, before immunity to malaria is acquired. There is now a

great deal of support for the hypothesis that the sickling trait protects against malaria, and in particular against *Plasmodium falciparum*, and this hypothesis is now widely accepted. The importance of malaria in maintaining the frequency of thalassaemia is not yet fully documented. In relation to the other common haemoglobin traits, only scanty data are available. This interesting aspect of the haemoglobin story is discussed in more detail by a number of authors (Allison, 1961; Motulsky, 1964a; Rucknagel and Neel, 1961; Livingstone, 1958; Allison, 1964).

The Distribution of Sickle Cell Trait. The distribution of the sickle cell gene is shown in Fig. 22. It can be seen that sickling occurs in tropical Africa and also in the Mediterranean region, southern Arabia, and India. Besides occurring in these areas, it is, of course, also found in emigré populations, such as the American and West Indian Negroes. The incidence of the gene varies enormously from place to place; the highest frequencies are found in certain peoples of East Africa, reaching 40% in Amba on the slopes of Mount Ruwenzori.

The distribution of the sickling gene, in general, corresponds well to that of *falciparum* malaria (Fig. 23). There are, however, areas where *falciparum* malaria is common and the sickling trait is not found, such as South-east Asia. The reasons for this are not entirely clear. It may be that the trait has never been introduced into these areas either by mutation or immigration. Alternatively, protection against malaria may be afforded by one of the other red cell traits. For example, in South-east Asia Hb-E, thalassaemia, and glucose 6-phosphate dehydrogenase (G6PD) deficiency all occur, and all are thought to give protection against malaria, the relatively more lethal sickle gene being excluded. This problem has been studied in Greece, where the sickle cell trait and thalassaemia are found. It was found that in regions where the sickle gene frequency is high, the frequency of thalassaemia is low and vice versa (Barnicot, Allison, Blumberg, Deliyannis, Krimbas, and Ballas, 1963). The theoretical aspects of this situation have been discussed by Penrose, Smith, and Sprott (1956).

It would be expected that considerable protection against death from malaria would be needed to balance the loss of sickle cell genes in regions where its frequency is high. The demonstration of this is one of the most vital links in the malaria hypothesis. In general, death rates from malaria are difficult to obtain, because it is often difficult to be sure that malaria, if present, is not just an incidental finding

FIG. 22. Frequency of the sickle cell gene Hb^S in various parts of the Old World (after Allison, 1961).

rather than the primary cause of death. Hence cerebral malaria provides the most favourable material for this kind of study. Several groups of workers have determined the incidence of sickling in infants dying from cerebral malaria and compared this with that found in the general population.

The results of these studies have been summarized by Motulsky (1964a) and are given in Table IV. This shows that whereas 23 out of the 100 patients dying from cerebral malaria would have been expected to carry the sickling trait, only one such patient was found, and this gives strong support to the hypothesis that the high incidence of sickling trait is due to its protection against death from falciparum malaria.

The Distribution of Haemoglobin

C. High frequencies of Hb-C are only found in certain regions of West Africa; the trait reaching a frequency of almost 30%. Besides this, sporadic cases are found elsewhere in the African continent. There is some evidence that in those areas where both Hb-S and Hb-C are found in the population, their frequencies are inversely related (Rucknagel and Neel, 1961). As might be expected, Hb-C is also found in the Negro populations of America and the West Indies.

The high frequency of Hb-C is also thought to be due to its protective effect against malaria, and this is supported, but by no means proved, by the few studies that have been carried out (Motulsky, 1964a). Hb-C disease is much milder than sickle cell disease and an equilibrium frequency of Hb-C

FIG. 23. Distribution of falciparum malaria before 1930 (from M. F. Boyd's *Malaria*).

TABLE IV
MALARIAL MORTALITY IN Hb-S TRAIT (AS) (From Motulsky, 1964a)

	% AS in Population	No. Dead From Malaria	Observed No. AS Dead From Malaria	Expected No. AS Dead From Malaria
Leopoldville, Congo (Lambotte-Legrande and Lambotte-Legrande, 1958)	26	23	0	6
Luluaburg, Congo (Vandepitte and Delaisse, 1957)	29	21	1	6.1
Ibadan, Nigeria (Edington and Watson-Williams, 1964)	24	27	0	6.5
Accra, Ghana (Edington and Watson-Williams, 1964)	8	13	0	1
Kampala, Uganda (Raper, 1956)	19	16	0	3
Total		100	1*	22.6

* $\chi^2 = 20.5$; $p < 0.001$.

could be maintained by a much smaller selective advantage of the heterozygote than that conferred by the sickle cell trait, making it correspondingly more difficult to demonstrate.

The Distribution of Haemoglobin D. Hb-D is found sporadically in many parts of the world. It occurs somewhat more commonly in certain regions of India but even there the maximum incidence of the trait is 2% in Sikhs of the Punjab (Bird and Lehmann, 1956). As has already been mentioned, detailed studies of samples of Hb-D have shown that there are several chemically distinct forms.

The Distribution of Haemoglobin E. This is shown in Fig. 24. It can be seen that Hb-E is found almost exclusively in South-east Asia, although cases have been reported to occur as far

away as Turkey (Aksoy and Lehmann, 1957). The distribution of the abnormal haemoglobins in South-east Asia has recently been reviewed by Lie Injo Luan Eng (1964). The reasons for the frequency of Hb-E in this region are not clear, but again protection against malaria has been suggested (Kruatrachue, Na-Nakorn, Charoenlarp, and Suwanakul, 1961; see Allison, 1961; Motulsky, 1964a).

The Distribution of Thalassaemia. The map shown in Fig. 25 does not differentiate between the distribution of α - and β -thalassaemia, but mainly represents that of the latter. α -thalassaemia is, however, known to occur in at least some of the areas in which β -thalassaemia is present and has been reported from Italy, Greece, Israel, and other Eastern Mediterranean countries, South-east Asia, and West Africa (see Motulsky, 1964b). Again, it has been suggested that protection against falc-

FIG. 24. Distribution of haemoglobin E (from Allison, 1961).

FIG. 25. Distribution of thalassaemia (from Allison, 1961).

parum malaria maintains the frequency of the thalassaemia traits, but the evidence for this is not nearly so convincing as in the case of the sickle cell trait.

We wish to thank Professor N. A. Barnicot for helpful discussion during the preparation of this review. The work was supported by grant NB-04270 from the National Institute of Health.

REFERENCES

- Ager, J. A. M., and Lehmann, H. (1958). Observations on some 'fast' haemoglobins: K, J, N, and 'Bart's'. *Brit. med. J.*, **1**, 929.
- Aksoy, M., and Lehmann, H. (1957). The first observation of sickle-cell haemoglobin E disease. *Nature (Lond.)*, **179**, 1248.
- Allen, D. W., Schroeder, W. A., and Balog, J. (1958). Observations on the chromatographic heterogeneity of normal adult and fetal human haemoglobin: a study of the effects of crystallization and chromatography on the heterogeneity and isoleucine content. *J. Amer. chem. Soc.*, **80**, 1628.
- Allison, A. C. (1954). The distribution of the sickle-cell trait in East Africa and elsewhere and its apparent relationship to the incidence of subtertian malaria. *Trans. roy. Soc. trop. Med. Hyg.*, **48**, 312.
- (1961). Abnormal haemoglobin and erythrocyte enzyme-deficiency traits. In *Genetical Variation in Human Populations*, ed. G. A. Harrison, p. 16. Pergamon Press, Oxford.
- (1964). Polymorphism and natural selection in human populations. *Cold Spr. Harb. Symp. quant. Biol.*, **29**. In the press.
- Anson, M. L., and Mirsky, A. E. (1930). Protein coagulation and its reversal; the preparation of insoluble globin, soluble globin and heme. *J. gen. Physiol.*, **13**, 469.
- Askonas, B. A., and Smyth, D. G. (1964). Antigenicity of the β -chain of human haemoglobin. *Nature (Lond.)*, **201**, 496.
- Atwater, J., Huehns, E. R., and Shooter, E. M. (1961). Haemoglobin I₁, a further variant of haemoglobin A₁. *J. molec. Biol.*, **3**, 707.
- , Schwartz, I. R., Erslev, A. J., Montgomery, T. L., and Tocantins, L. M. (1960a). Sickling of erythrocytes in a patient with thalassaemia-hemoglobin-I disease. *New Engl. J. Med.*, **263**, 1215.
- , —, and Tocantins, L. M. (1960b). A variety of human hemoglobin with 4 distinct electrophoretic components. *Blood*, **15**, 901.
- Babin, D. R., Jones, R. T., and Schroeder, W. A. (1964). Haemoglobin D_{Los Angeles} $\alpha_1^A\beta_1^{21}\text{GluNH}_1$. *Biochim. biophys. Acta (Amst.)*, **86**, 136.
- Bachmann, F., and Marti, H. R. (1962). Hemoglobin Zürich. II. Physicochemical properties of the abnormal hemoglobin. *Blood*, **20**, 272.
- Baglioni, C. (1961). An improved method for the fingerprinting of human hemoglobin. *Biochim. biophys. Acta (Amst.)*, **48**, 392.
- (1962a). Abnormal human haemoglobins. VIII. Chemical studies on haemoglobin D. *ibid.*, **59**, 437.
- (1962b). The fusion of two peptide chains in hemoglobin Lepore and its interpretation as a genetic deletion. *Proc. nat. Acad. Sci. (Wash.)*, **48**, 1880.
- (1962c). A chemical study of hemoglobin Norfolk. *J. biol. Chem.*, **237**, 69.
- (1963a). Correlations between genetics and chemistry of human hemoglobins. In *Molecular Genetics*, ed. J. H. Taylor, Pt. 1, p. 405. Academic Press, New York.
- (1963b). A child homozygous for persistence of fetal haemoglobin. *Nature (Lond.)*, **198**, 1177.
- , and Ingram, V. M. (1961). Abnormal human hemoglobins. V. Clinical investigation of haemoglobins A, G, C, X from one individual. *Biochim. biophys. Acta (Amst.)*, **48**, 253.
- , and Lehmann, H. (1962). Chemical heterogeneity of haemoglobin O. *Nature (Lond.)*, **196**, 229.
- , and Weatherall, D. J. (1963). Abnormal human hemoglobins. IX. Chemistry of hemoglobin J_{Baltimore}. *Biochim. biophys. Acta (Amst.)*, **78**, 637.
- Bannerman, R. M. (1961). *Thalassaemia—A Survey of Some Aspects*. Grune and Stratton, New York.
- , Grinstein, M., and Moore, C. V. (1959). Haemoglobin synthesis in thalassaemia; *in-vitro* studies. *Brit. J. Haemat.*, **5**, 102.
- Barkhan, P., and Adinolfi, M. (1962). Observations on the high foetal haemoglobin gene and its interaction with the thalassaemia gene. *J. clin. Path.*, **15**, 350.

- , Stevenson, M. E., Pinker, G., Dance, N., and Shooter, E. M. (1964). Haemoglobin Lepore trait: an analysis of the abnormal haemoglobin. *Brit. J. Haemat.*, **10**, 437.
- Barnabas, J., and Muller, C. J. (1962). Haemoglobin-LeporeHollandia. *Nature (Lond.)*, **194**, 931.
- Barnicot, N. A., Allison, A. C., Blumberg, B. S., Deliyannis, G., Krimbas, C., and Ballas, A. (1963). Haemoglobin types in Greek populations. *Ann. hum. Genet.*, **26**, 229.
- Bayrakci, C., Josephson, A., Singer, L., Heller, P., and Coleman, R. D. (1964). A new fast haemoglobin. *Proc. int. Soc. Haemat.*, **10**. In the press.
- Beale, D., and Lehmann, H. (1964). Haemoglobin J_{Oxford}. *Biochem. J.*, **93**, 8.
- Beaven, G. H., Ellis, M. J., and White, J. C. (1960a). Studies on human foetal haemoglobin. I. Detection and estimation. *Brit. J. Haemat.*, **6**, 1.
- , —, and — (1960b). Studies on human foetal haemoglobin. II. Foetal haemoglobin levels in healthy children and adults and in certain haematological disorders. *ibid.*, **6**, 201.
- , and Gratz, W. B. (1959). A critical review of human haemoglobin variants. *J. clin. Path.*, **12**, 1, 101.
- , Stevens, B. L., Shooter, E. M., Ellis, M. J., White, J. C., and Gillespie, J. E. O'N. (1964). An abnormal haemoglobin (Lepore/Cyprus) resembling haemoglobin-Lepore and its interaction with thalassaemia. *Brit. J. Haemat.*, **10**, 159.
- , Stevens, B. L., Dance, N., and White, J. C. (1963). Occurrence of haemoglobin H in leukaemia. *Nature (Lond.)*, **199**, 1297.
- Beet, E. A. (1949). Genetics of the sickle-cell trait in a Bantu tribe. *Ann. Eugen. (Lond.)*, **14**, 279.
- Benzer, S., Ingram, V. M., and Lehmann, H. (1958). Three varieties of human haemoglobin D. *Nature (Lond.)*, **182**, 852.
- Bergren, W. R., and Sturgeon, P. (1960). Hemoglobin H. Some additional findings. *Proc. int. Soc. Haemat.*, **7**, (2), part 1, 488.
- Betke, K., Marti, H. R., and Schlicht, I. (1959). Estimation of small percentages of foetal haemoglobin. *Nature (Lond.)*, **184**, 1877.
- Bianco, I., Modiano, G., Bottini, E., and Lucci, R. (1963). Alteration in the α -chain of haemoglobin L_{Perrara}. *ibid.*, **198**, 395.
- Bingle, J. P., Huehns, E. R., and Pranker, T. A. J. (1958). Haemoglobin-H disease. *Brit. med. J.*, **2**, 1389.
- Bird, G. W. G., and Lehmann, H. (1956). Haemoglobin D in India. *ibid.*, **1**, 514.
- Bishop, J., Leahy, J., and Schweet, R. (1960). Formation of the peptide chain of hemoglobin. *Proc. nat. Acad. Sci. (Wash.)*, **46**, 1030.
- Boulard, C., Cosset, A., Destaing, F., Duzer, A., Jonxis, J. H. P., Muller, C. J., and Portier, A. (1961). Hb I trait in an Algerian Musulman family, presenting two abnormal components. *Blood*, **18**, 750.
- Bowman, B. H., Oliver, C. P., Barnett, D. R., Cunningham, J. E., and Schneider, R. G. (1964). Chemical characterization of three hemoglobins G. *ibid.*, **23**, 193.
- Boyer, S. H., Rucknagel, D. L., Weatherall, D. J., and Watson-Williams, E. J. (1963). Further evidence for linkage between the β and δ loci governing human hemoglobin and the population dynamics of linked genes. *Amer. J. hum. Genet.*, **15**, 438.
- Bradley, T. B., Jr., Brawner, J. N., III, and Conley, C. L. (1961). Further observations on an inherited anomaly characterized by persistence of fetal hemoglobin. *Bull. Johns Hopk. Hosp.*, **108**, 242.
- Braunitzer, G., Gehring-Müller, G., Hilschmann, N., Hilse, K., Hobom, G., Rudloff, V., and Wittmann-Liebold, B. (1961). Die Konstitution des normalen adulten Humanhäoglobins. *Hoppe-Seyler's Z. physiol. Chem.*, **325**, 283.
- Bridges, J. M., Neill, D. W., and Lehmann, H. (1961). Increase in Hb-A₁ appearing after homograft of foetal haemopoietic tissue. *Brit. med. J.*, **1**, 1349.
- Bromberg, Y. M., Salzberger, M., and Abrahamov, A. (1957). Alkali resistant type of hemoglobin in women with molar pregnancy. *Blood*, **12**, 1122.
- Burnham, B. F., and Lascelles, J. (1963). Control of porphyrin biosynthesis through a negative-feedback mechanism. Studies with preparations of δ -aminolaevulinate synthetase and δ -aminolaevulinate dehydratase from *Rhodospseudomonas spheroides*. *Biochem. J.*, **87**, 462.
- Cepellini, R. (1959). In *Ciba Foundation Symposium on Biochemistry of Human Genetics*, p. 133, eds G. E. W. Wolstenholme and C. M. O'Connor. Churchill, London.
- Chernoff, A. I. (1953a). Immunologic studies of hemoglobins. I. The production of anti-hemoglobin sera and their immunologic characteristics. *Blood*, **8**, 399.
- (1953b). Immunologic studies of hemoglobins. II. Quantitative precipitin test using anti-fetal hemoglobin sera. *ibid.*, **8**, 413.
- (1961). Chromium-51 tagging of the α -chain of human haemoglobin. *Nature (Lond.)*, **192**, 327.
- (1964). Hb-A₄. A naturally occurring hemoglobin possessing only α -chains. *Proc. int. Soc. Haemat.*, **10**. In the press.
- , and Liu, J. C. (1961). The amino acid composition of hemoglobin. II. Analytical technics. *Blood*, **17**, 54.
- , and Perillie, P. E. (1964). The amino acid composition of Hgb New Haven 2 (Hgb N_{New Haven}). *Biochem. biophys. Res. Commun.*, **16**, 368.
- Clegg, M. D., and Schroeder, W. A. (1959). A chromatographic study of the minor components of normal adult human hemoglobin, including a comparison of hemoglobin from normal and phenylketonuric individuals. *J. Amer. chem. Soc.*, **81**, 6065.
- Conley, C. L., Weatherall, D. J., Richardson, S. N., Shepard, M. K., and Charache, S. (1963). Hereditary persistence of fetal hemoglobin: a study of 79 affected persons in 15 negro families in Baltimore. *Blood*, **21**, 261.
- Crick, F. H. C., Barnett, L., Brenner, S., and Watts-Tobin, R. J. (1961). General nature of the genetic code for proteins. *Nature (Lond.)*, **192**, 1227.
- Cullis, A. F., Muirhead, H., Perutz, M. F., Rossmann, M. G., and North, A. C. T. (1962). The structure of hemoglobin. IX. A three-dimensional Fourier synthesis at 5.5 Å resolution: description of the structure. *Proc. roy. Soc. Lond., Series A*, **265**, 161.
- Dacie, J. V., Grimes, A. J., Meisler, A., Steingold, L., Hemsted, E. H., Beaven, G. H., and White, J. C. (1964). Hereditary Heinz-body anaemia: a report of studies on five patients with mild anaemia. *Brit. J. Haemat.*, **10**, 388.
- Dance, N., and Huehns, E. R. (1962). A haemoglobin containing only δ -chains. *Biochem. biophys. Res. Commun.*, **7**, 444.
- , —, and Beaven, G. H. (1963). The abnormal haemoglobins in haemoglobin-H disease. *Biochem. J.*, **87**, 240.
- , —, and Shooter, E. M. (1964). The chemical investigation of haemoglobins G_{Bristol} and G_{Bristol/C}. *Biochim. biophys. Acta (Amst.)*, **86**, 144.
- De Vries, A., Joshua, H., Lehmann, H., Hill, R. L., and Fellow, R. E. (1963). The first observation of an abnormal haemoglobin in a Jewish family: haemoglobin Beilinson. *Brit. J. Haemat.*, **9**, 484.
- Diggs, L. W., and Bibb, J. (1939). The erythrocyte in sickle cell anemia. *J. Amer. med. Ass.*, **112**, 695.
- Dintzis, H. M. (1961). Assembly of peptide chains of hemoglobin. *Proc. nat. Acad. Sci. (Wash.)*, **47**, 247.
- Dormandy, K. M., Lock, S. P., and Lehmann, H. (1961). Haemoglobin Q-alpha-thalassaemia. *Brit. med. J.*, **1**, 1582.
- Drabkin, D. L., and Wise, C. D. (1962). Creation of a crystalline interspecies hybrid of hemoglobin at pH close to neutrality. *J. biol. Chem.*, **237**, 261.
- Drescher, H., and Künzer, W. (1954). Der Blutfarbstoff des menschlichen Feten. *Klin. Wschr.*, **32**, 92.
- Edington, G. M., and Lehmann, H. (1955a). Expression of the sickle-cell gene in Africa. *Brit. med. J.*, **1**, 1308.
- , and — (1955b). Expression of the sickle-cell gene in Africa. *ibid.*, **2**, 1328.
- , and Watson-Williams, E. J. (1964). Sickling, haemoglobin C, glucose-6-phosphate dehydrogenase deficiency and malaria in Western Nigeria. *C.I.O.M.S. Symposium on Abnormal Haemoglobins and Enzyme Deficiency*. Blackwell, Oxford. In the press.
- Fessas, P. (1960). Observations on a second haemoglobin abnormality in haemoglobin-H disease. *Proc. 7th Congr. europ. Soc. Haemat.*, part II, 1043.
- (1962). Haemoglobin H and Bart's. In *Haemoglobin Colloquium*, Vienna, 1961, ed. H. Lehmann and K. Betke, p. 74. Thieme, Stuttgart.
- (1964). Forms of thalassaemia. In *C.I.O.M.S. Symposium on Abnormal Haemoglobins and Enzyme Deficiency*. Blackwell, Oxford. In the press.
- , Karaklis, A., and Gnafakis, N. (1961). A further abnormality of foetal haemoglobin. *Acta Haemat. (Basel)*, **25**, 62.
- , and Loukopoulos, D. (1964). Alpha-chain of human hemoglobin: occurrence in vivo. *Science*, **143**, 590.

- , Mastrokalos, N., and Fostiropoulos, G. (1959). New variant of human foetal haemoglobin. *Nature (Lond.)*, **183**, 30.
- , and Papaspyrou, A. (1957). New 'fast' hemoglobin associated with thalassemia. *Science*, **126**, 1119.
- , and Stamatoyannopoulos, G. (1962). Absence of haemoglobin A₁ in an adult. *Nature (Lond.)*, **195**, 1215.
- , and — (1964). Hereditary persistence of fetal hemoglobin in Greece. A study and a comparison. *Blood*, **24**, 223.
- , —, and Karaklis, A. (1962). Hemoglobin 'Pylos': study of a hemoglobinopathy resembling thalassemia in the heterozygous, homozygous and double heterozygous state. *ibid.*, **19**, 1.
- Frick, P. G., Hitzig, W. H., and Betke, K. (1962). Hemoglobin Zürich. I. A new hemoglobin anomaly associated with acute hemolytic episodes with inclusion bodies after sulfonamide therapy. *ibid.*, **20**, 261.
- Gammack, D. B., Huehns, E. R., Lehmann, H., and Shooter, E. M. (1961). The abnormal polypeptide chains in a number of haemoglobin variants. *Acta Genet. (Basel)*, **11**, 1.
- , —, Shooter, E. M., and Gerald, P. S. (1960). Identification of the abnormal polypeptide chain of hemoglobin G₁₀. *J. molec. Biol.*, **2**, 372.
- Gerald, P. S. (1958). The electrophoretic and spectroscopic characterization of Hgb M. *Blood*, **13**, 936.
- , and Diamond, L. K. (1958a). The diagnosis of thalassemia trait by starch block electrophoresis of the hemoglobin. *ibid.*, **13**, 61.
- , and — (1958b). A new hereditary hemoglobinopathy (the Lepore trait) and its interaction with thalassemia trait. *ibid.*, **13**, 835.
- , and Efron, M. L. (1961). Chemical studies of several varieties of Hb. M. *Proc. nat. Acad. Sci. (Wash.)*, **47**, 1758.
- , and George, P. (1959). Second spectroscopically abnormal methemoglobin associated with hereditary cyanosis. *Science*, **129**, 393.
- , and Ingram, V. M. (1961). Recommendations for the nomenclature of hemoglobins. *J. biol. Chem.*, **236**, 2155.
- Gibson, Q. H. (1948). Reduction in methaemoglobin in red blood cells and studies on the cause of idiopathic methaemoglobinemia. *Biochem. J.*, **42**, 13.
- Gottlieb, A. J., Restrepo, A., and Itano, H. A. (1964). Hb J^{medellin}. Chemical and genetic study. *Fed. Proc.*, **23**, 172.
- Gouttas, A., Fessas, P., Tseveris, H., and Xefteri, E. (1955). Description d'une nouvelle variété d'anémie hémolytique congénitale: étude hématologique électrophorétique et génétique. *Sang*, **26**, 911.
- Gray, S. J., and Sterling, K. (1950). The tagging of red cells and plasma proteins with radioactive chromium. *J. clin. Invest.*, **29**, 1604.
- Griggs, R. C., and Harris, J. W. (1955). The biophysics of the variants of sickle cell disease. *ibid.*, **34**, 937.
- Grimes, A. J., and Meisler, A. (1962). Possible cause of Heinz bodies in congenital Heinz-body anaemia. *Nature (Lond.)*, **194**, 190.
- , —, and Dacie, J. V. (1964). Congenital Heinz-body anaemia: further evidence on the cause of Heinz-body production in red cells. *Brit. J. Haemat.*, **10**, 281.
- Guidotti, G., Konigsberg, W., and Craig, L. C. (1963). On the dissociation of normal adult human hemoglobin. *Proc. nat. Acad. Sci. (Wash.)*, **50**, 774.
- Hahn, E. V., and Gillespie, E. B. (1927). Sickle cell anemia: a report of a case greatly improved by splenectomy. *Arch. intern. Med.*, **39**, 233.
- Haldane, J. B. S. (1949). Disease and evolution. *La Ricerca Scientif. (suppl.)*, **19**, 68.
- Hall-Craggs, M., Marsden, P. D., Raper, A. B., Lehmann, H., and Beale, D. (1964). Homozygous sickle-cell anaemia arising from two different haemoglobins S. Interaction of haemoglobin S and Stanleyville-II. *Brit. med. J.*, **2**, 87.
- Ham, T. H., and Castle, W. B. (1940). Relation of increased hypotonic fragility and of erythrocytosis to the mechanism of hemolysis in certain anemias. *Trans. Ass. Amer. Physns*, **55**, 127.
- Hamilton, H. E., Sheets, R. F., and Brosseau, G. (1962). Gamma thalassemia. *J. Lab. clin. Med.*, **60**, 880.
- Hanada, M., and Rucknagel, D. L. (1963). The abnormality of the primary structure of hemoglobin Shimonoseki. *Biochem. biophys. Res. Commun.*, **11**, 229.
- Harris, J. W., Brewster, H. H., Ham, T. H., and Castle, W. B. (1956). Studies on the destruction of red blood cells. X. The biophysics and biology of sickle-cell disease. *Arch. int. Med.*, **97**, 145.
- Hayashi, H. (1961). A simple method for the fractionation of globins into their α - and β -chains. *J. Biochem. (Tokyo)*, **50**, 70.
- Heck, W., and Wolf, H. (1958). Angeborener Herzfehler mit Cyanose durch pathologischen Blutfarbstoff (Hämoglobin M). *Ann. paediat. (Basel)*, **190**, 135.
- Heisterkamp, D., and Ebaugh, F. G., Jr. (1962). Site of attachment of the chromate ion to the haemoglobin molecule. *Nature (Lond.)*, **193**, 1253.
- Heller, P., Weinstein, H. G., Yakulis, V. J., and Rosenthal, I. M. (1962b). Haemoglobin M^{Kankakee}, a new variant of haemoglobin M. *Blood*, **20**, 287.
- , Yakulis, V. J., Epstein, R. B., and Friedland, S. (1963). Variation in the amount of hemoglobin S in a patient with sickle cell trait and megaloblastic anemia. *ibid.*, **21**, 479.
- , —, and Josephson, A. M. (1962a). Immunologic studies of human hemoglobins. *J. Lab. clin. Med.*, **59**, 401.
- Herman, E. C., Jr., and Conley, C. L. (1960). Hereditary persistence of fetal hemoglobin. A family study. *Amer. J. Med.*, **29**, 9.
- Hill, R. J., and Craig, L. C. (1959). Countercurrent distribution studies with adult human hemoglobin. *J. Amer. chem. Soc.*, **81**, 2272.
- , and Kraus, A. P. (1963). Studies on the amino acid sequence of Hb A₂. *Fed. Proc.*, **22**, 597.
- Hill, R. L. (1961). The abnormal human hemoglobins. *Lab. Invest.*, **10**, 1012.
- , Swenson, R. T., and Schwartz, H. C. (1960). Characterization of a chemical abnormality in hemoglobin G. *J. biol. Chem.*, **235**, 3182.
- Holmquist, W. R., and Schroeder, W. A. (1964). Properties and partial characterization of adult hemoglobin A_{1c}. *Biochim. biophys. Acta (Amst.)*, **82**, 639.
- Hörlein, H., and Weber, G. (1948). Über chronische familiäre Methämoglobinämie und eine neue Modifikation des Methämoglobins. *Dtsch. med. Wschr.*, **73**, 476.
- , and — (1951). Chronische familiäre Methämoglobinämie. *Z. ges. inn. Med.*, **6**, 197.
- Horton, B., Payne, R. A., Bridges, M. T., and Huisman, T. H. J. (1961). Studies on an abnormal minor hemoglobin component (Hb-B₃). *Clin. chim. Acta*, **6**, 246.
- Horton, B. F., and Huisman, T. H. J. (1963). Linkage of the β -chain and δ -chain structural genes of human hemoglobins. *Amer. J. hum. Genet.*, **15**, 394.
- Huehns, E. R. (1962). *Haemoglobin H Disease. Clinical and Experimental Studies*. M.D. Thesis, University of London.
- , Beaven, G. H., and Stevens, B. L. (1964e). Reaction of haemoglobin α^A with haemoglobins β^A , γ^A , and δ^A . *Biochem. J.*, **92**, 444.
- , —, and — (1964f). Recombination studies on haemoglobins at neutral pH. *ibid.*, **92**, 440.
- , Dance, N., Beaven, G. H., Hecht, F., and Motulsky, A. G. (1964b). Human embryonic haemoglobins. *Cold Spr. Harb. Symp. quant. Biol.*, **29**. In the press.
- , —, Keil, J. V., Hecht, F., and Motulsky, A. G. (1964a). Human embryonic haemoglobins. *Nature (Lond.)*, **201**, 1095.
- , —, Shooter, E. M., and Beaven, G. H. (1962b). The preparation and properties of haemoglobin α^A , a haemoglobin consisting of α^A -polypeptide chains. *J. molec. Biol.*, **5**, 511.
- , Flynn, F. V., Butler, E. A., and Beaven, G. H. (1961a). Two new haemoglobin variants in a very young human embryo. *Nature (Lond.)*, **189**, 496.
- , —, —, and Shooter, E. M. (1960). The occurrence of haemoglobin 'Bart's' in conjunction with haemoglobin H. *Brit. J. Haemat.*, **6**, 388.
- , Hecht, F., Keil, J. V., and Motulsky, A. G. (1964c). Developmental hemoglobin anomalies in a chromosomal triplication: D, trisomy syndrome. *Proc. nat. Acad. Sci. (Wash.)*, **51**, 89.
- , and Shooter, E. M. (1961a). Polypeptide chains of haemoglobin A₂. *Nature (Lond.)*, **189**, 918.
- , and — (1961b). The polypeptide chains of haemoglobin A₂ and haemoglobin-G₁₀. *J. molec. Biol.*, **3**, 257.
- , and — (1962). Reaction of haemoglobin α^A with haemoglobin H. *Nature (Lond.)*, **193**, 1083.

- , and — (1964a). Haemoglobin. *Sci. Progr.*, **52**, 353.
- , and — (1964b). The subunits of haemoglobins. 1st Meeting of the Federation of European Biochemical Societies, London. 1964 Symposium. The Structure and Activity of Enzymes. 167.
- , —, and Beaven, G. H. (1962a). On the recombination of canine and human haemoglobins. *J. molec. Biol.*, **4**, 323.
- , —, and — (1964d). The time course of the recombination of human adult and canine haemoglobins. *Biochem. J.*, **91**, 331.
- , —, Dance, N., Beaven, G. H., and Shooter, K. V. (1961b). Haemoglobin α^A . *Nature (Lond.)*, **192**, 1057.
- Huisman, T. H. J. (1960). Genetic aspects of two different minor haemoglobin components found in cord blood samples of Negro babies. *ibid.*, **188**, 589.
- (1962). A new alpha chain-abnormal hemoglobin in a white family. In *Haemoglobin Colloquium, Vienna 1961*, ed. H. Lehmann and K. Betke, p. 37. Thieme, Stuttgart.
- , and Dozy, A. M. (1962). Studies on the heterogeneity of hemoglobin. IV. Chromatographic behaviour of different human haemoglobins on anion-exchange cellulose (DEAE cellulose). *J. Chromatog.*, **7**, 180.
- , Martis, E. A., and Dozy, A. (1958). Chromatography of hemoglobin types of carboxymethylcellulose. *J. Lab. clin. Med.*, **52**, 312.
- , and Meyering, C. A. (1960). Studies on the heterogeneity of hemoglobin. I. The heterogeneity of different human hemoglobin types in carboxymethylcellulose and in Amberlite IRC-50 chromatography: qualitative aspects. *Clin. chim. Acta*, **5**, 103.
- , and Prins, H. K. (1955). Chromatographic estimation of four different human hemoglobins. *J. Lab. clin. Med.*, **46**, 255.
- , Punt, K., and Schaad, J. D. G. (1961). Thalassemia minor associated with hemoglobin-B₂ heterozygosity. A family report. *Blood*, **17**, 747.
- , and Sydenstricker, V. P. (1962). Difference in gross structure of two electrophoretically identical 'minor' haemoglobin components. *Nature (Lond.)*, **193**, 489.
- Hunt, J. A. (1959). Identity of the α -chains of adult and foetal human haemoglobins. *ibid.*, **183**, 1373.
- , and Ingram, V. M. (1958). Abnormal human haemoglobins. II. The chymotryptic digestion of the trypsin-resistant 'core' of haemoglobins A and S. *Biochim. biophys. Acta (Amst.)*, **28**, 546.
- , and — (1960). Abnormal human haemoglobins. IV. The chemical difference between normal human haemoglobin and haemoglobin C. *ibid.*, **42**, 409.
- , and — (1961). Abnormal human haemoglobins. VI. The chemical difference between haemoglobins A and E. *ibid.*, **49**, 520.
- , and Lehmann, H. (1959). Haemoglobin 'Bart's': a foetal haemoglobin without α -chains. *Nature (Lond.)*, **184**, 872.
- Hutchison, H. E., Pinkerton, P. H., Waters, P., Douglas, A. S., Lehmann, H., and Beale, D. (1964). Hereditary Heinz-body anaemia, thrombocytopenia, and haemoglobinopathy (Hb Köln) in a Glasgow family. *Brit. med. J.*, **2**, 1099.
- Hynes, M., and Lehmann, H. (1956). Haemoglobin D in a Persian girl: presumably the first case of haemoglobin-D-thalassaemia. *ibid.*, **2**, 923.
- Ingram, V. M. (1956). A specific chemical difference between the globins of normal human and sickle-cell anaemia haemoglobin. *Nature (Lond.)*, **178**, 792.
- (1958). Abnormal human haemoglobins. I. The comparison of normal human and sickle-cell haemoglobins by 'fingerprinting'. *Biochim. biophys. Acta (Amst.)*, **28**, 539.
- (1959). Separation of the peptide chains of human globin. *Nature (Lond.)*, **183**, 1795.
- (1961). *Hemoglobin and its Abnormalities*. Charles C. Thomas, Springfield, Illinois.
- (1964). A molecular model for thalassaemia. *Ann. N.Y. Acad. Sci.* In the press.
- , and Stretton, A. O. W. (1959). Genetic basis of the thalassaemia diseases. *ibid.*, **184**, 1903.
- , and Stretton, A.O.W. (1961). Human haemoglobin A₂: chemistry, genetics and evolution. *ibid.*, **190**, 1079.
- , and — (1962a). Human haemoglobin A₁. I. Comparison of haemoglobins A₁ and A. *Biochim. biophys. Acta (Amst.)*, **62**, 456.
- , and — (1962b). Human haemoglobin A₁. II. The chemistry of some peptides peculiar to haemoglobin A₁. *ibid.*, **63**, 20.
- Itano, H. A. (1953). Qualitative and quantitative control of adult hemoglobin synthesis—a multiple allele hypothesis. *Amer. J. hum. Genet.*, **5**, 34.
- (1957). The human hemoglobins: their properties and genetic control. *Advanc. Protein Chem.*, **12**, 215.
- (1964). The synthesis and structure of normal and abnormal hemoglobins. In *C.I.O.M.S. Symposium on Abnormal Haemoglobins and Enzyme Deficiency*. Blackwell, Oxford. In the press.
- , Bergren, W. R., and Sturgeon, P. (1956). The abnormal human hemoglobins. *Medicine (Baltimore)*, **35**, 121.
- , and Robinson, E. (1959). Properties and inheritance of haemoglobin by asymmetric recombination. *Nature (Lond.)*, **184**, 1468.
- , and — (1960). Genetic control of the α - and β -chains of hemoglobin. *Proc. nat. Acad. Sci. (Wash.)*, **46**, 1492.
- , and Singer, S. J. (1958). Dissociation and recombination of human adult hemoglobins A, S and C. *ibid.*, **44**, 522.
- , and Robinson, E. (1959). Abnormal and genetical units of the haemoglobin molecule. In *Ciba Found. Symp. Biochemistry of Human Genetics*, ed. G. E. W. Wolstenholme and C. M. O'Connor, p. 96. Churchill, London.
- Jaffe, E. R., and Heller, P. (1964). Methemoglobinemia in man. *Progr. Hemat.*, **4**, 48.
- Jacob, F., and Monod, J. (1961). Genetic regulatory mechanisms in the synthesis of proteins. *J. Mole. Biol.*, **3**, 318.
- Jacob, G. F., and Raper, A. B. (1958). Hereditary persistence of foetal haemoglobin production, and its interaction with the sickle-cell trait. *Brit. J. Haemat.*, **4**, 138.
- Jones, J. H. (1961). Foetal haemoglobin in Fanconi type anaemia. *Nature (Lond.)*, **192**, 982.
- Jones, R. T. (1964). Structural studies of amino ethylated hemoglobins by automatic peptide chromatography. *Cold Spr. Harb. Symp. Quant. Biol.*, **29**. In the press.
- , Coleman, R. D., and Heller, P. (1964). The chemical structure of hemoglobin M_I and M_K. *Fed. Proc.*, **23**, 173.
- , Koler, R. D., and Lisker, R. (1963). The chemical structure of hemoglobin Mexico determined by automatic peptide chromatography and subunit hybridization. *Clin. Res.*, **11**, 105.
- , and Schroeder, W. A. (1963). Chromatography of human hemoglobin. Factors influencing chromatography and differentiation of similar hemoglobins. *J. Chromat.*, **10**, 421.
- , —, Balog, J. E., and Vinograd, J. R. (1959). Gross structure of hemoglobin H. *J. Amer. chem. Soc.*, **81**, 3161.
- Jonxis, J. H. P. (1951). Personal communication. In *The Physiology of the Newborn Infant*, by C. A. Smith, 2nd ed., p. 9. Blackwell, Oxford.
- (1961). Über hämoglobinopathien mit besonderer Berücksichtigung der Cooley-Anämie. *Schweiz. med. Wschr.*, **91**, 1037.
- (1963). Hemoglobinopathies. *Ann. Rev. Med.*, **14**, 297.
- Joje, E. M. (1949). The ultraviolet spectral absorption of haemoglobin inside and outside the red blood cell. In *Haemoglobin (Barcroft Memorial Conference)*, ed. F. J. W. Roughton and J. C. Kendrew, p. 205. Butterworth, London.
- Josephson, A. M., Masri, M. S., Singer, L., Dworkin, D., and Singer, K. (1958). Starch block electrophoretic studies of human hemoglobin solutions. II. Results in cord blood, thalassemia and other hematologic disorders: comparison with Tiselius electrophoresis. *Blood*, **13**, 543.
- , Weinstein, H. G., Yakulis, V. J., Singer, L., and Heller, P. (1962). A new variant of hemoglobin M disease: hemoglobin M_{Chicago}. *J. Lab. clin. Med.*, **59**, 918.
- Karaklis, A., and Fessas, P. (1963). Normal minor components of human foetal haemoglobin. *Acta haemat. (Basel)*, **29**, 267.
- Kekwick, R. A., and Lehmann, H. (1960). Sedimentation characteristics of the γ -chain haemoglobin (haemoglobin 'Bart's'). *Nature (Lond.)*, **187**, 158.
- Kochhar, B. R., and Kathalia, P. M. L. (1963). Hemoglobin E-thalassaemia disease. *Indian J. med. Sci.*, **17**, 138.
- Konigsberg, W., and Hill, R. J. (1962). The structure of human hemoglobin. III. The sequence of amino acids in the tryptic peptides of the α -chain. *J. biol. Chem.*, **237**, 2547.
- Körber, E. (1866). Ueber Differenzen des Blutfarbstoffe. Inaugural Dissertation, Dorpat, cited by J. C. White and G. H. Beaven (1959). *Brit. med. Bull.*, **15**, 33.
- Kraus, A. P., Koch, B., and Burckett, L. (1961). Two families showing interaction of haemoglobin C or thalassaemia with high foetal haemoglobin in adults. *Brit. med. J.*, **1**, 1434.

- Kruatrachue, M., Na-Nakorn, S., Charoenlarp, P., and Suwanakul, L. (1961). Haemoglobin E and malaria in south-east Thailand. *Ann. trop. Med. Parasit.*, **55**, 468.
- Kunkel, H. G. (1954). Zone electrophoresis. *Meth. biochem. Anal.*, p. 141.
- , and Bearn, A. G. (1957). Minor hemoglobin components of normal human blood. *Fed. Proc.*, **16**, 760.
- , Ceppellini, R., Müller-Eberhard, U., and Wolf, J. (1957). Observations on the minor basic hemoglobin component in the blood of normal individuals and patients with thalassemia. *J. clin. Invest.*, **36**, 1615.
- , and Wallenius, G. (1955). New hemoglobin in normal adult blood. *Science*, **122**, 288.
- Labie, D., Rosa, J., Dreyfus, J. C., and Shapira, G. (1962). Inter-specific hybridization of haemoglobin. *Nature (Lond.)*, **194**, 384.
- Lambotte-Legrande, J., and Lambotte-Legrande, C. (1958). Notes complémentaires sur la drépanocytose. II. Sicklémie et anémie par ankylostomiasis. *Ann. Soc. belge Méd. trop.*, **38**, 55.
- Lange, R. D., and Akeroyd, J. H. (1958). Congenital hemolytic anemia with abnormal pigment metabolism and red cell inclusion bodies: a new clinical syndrome. *Blood*, **13**, 950.
- , Minnich, V., and Moore, C. V. (1951). Effect of oxygen tension and of pH on the sickling and mechanical fragility of erythrocytes from patients with sickle cell anemia and sickle cell trait. *J. Lab. clin. Med.*, **37**, 789.
- Lascelles, J. (1960). The synthesis of enzymes concerned in bacteriochlorophyll formation in growing cultures of *Rhodospseudomonas spheroides*. *J. gen. Microbiol.*, **23**, 487.
- Lehmann, H. (1960). The laboratory detection of abnormal haemoglobins. *Ass. clin. Path. Broadsheets No. 27*.
- , and Ager, J. A. M. (1960). *The Metabolic Basis of Inherited Disease*, p. 1086. McGraw-Hill, New York.
- , Beale, D., and Boi-Doku, F. S. (1964). Haemoglobin G_Accra. *Nature (Lond.)*, **203**, 363.
- , Story, P., and Thein, H. (1956). Haemoglobin E in Burmese. Two cases of haemoglobin E disease. *Brit. med. J.*, **1**, 544.
- Levin, W. C. (1958). 'Asymptomatic' sickle cell trait. *Blood*, **13**, 904.
- Lie-Injo Luan Eng (1961) Haemoglobin 'Bart's' and the sickling phenomenon. *Nature (Lond.)*, **191**, 1314.
- (1962). Alpha-chain thalassemia and hydrops fetalis in Malaya: report of five cases. *Blood*, **20**, 581.
- (1964). Haemoglobinopathies in East Asia. *Ann. hum. Genet.*, **28**, 101.
- , and Hart, P. L. de V. (1963). Splenectomy in two cases of haemoglobin Q-H-disease (Hb Q- α -thalassaemia). *Acta haemat. (Basel)*, **29**, 358.
- , and Jo Bwan Hie (1960). Hydrops foetalis with a fast-moving haemoglobin. *Brit. med. J.*, **2**, 1649.
- , and Lie Hong Gie (1961). Abnormal haemoglobin production as a probable cause of erythroblastosis and hydrops foetalis in uniovular twins. *Acta haemat. (Basel)*, **25**, 192.
- Livingstone, F. B. (1958). Anthropological implications of sickle cell gene distribution in West Africa. *Amer. Anthropol.*, **60**, 633.
- London, I. (1964). The synthesis of haemoglobin. *Proc. Int. Soc. Haemat.*, **10**. In the press.
- Malamos, B., Fessas, P., and Stamatoyannopoulos, G. (1962). Types of thalassaemia-trait carriers as revealed by a study of their incidence in Greece. *Brit. J. Haemat.*, **8**, 5.
- Malcolm, D., Ranney, H. M., and Jacobs, A. S. (1963). Association of radioactive chromium with various components of hemoglobin. *Blood*, **21**, 8.
- Marks, P. A., and Burka, E. R. (1964). Hemoglobin A and F: formation in thalassemia and other hemolytic anemias. *Science*, **144**, 552.
- Marti, H. R. (1963). *Normale und anormale menschliche Hämoglobine*, p. 85. Springer, Berlin.
- , and Büttler, R. (1961). Hämoglobin F-und hämoglobin A₁-Vermehrung bei der Schweizer Bevölkerung. *Acta haemat. (Basel)*, **26**, 65.
- Mathur, K. S., and Misra, G. M. (1962). An unstable haemoglobin associated with myeloid leukaemia (a case report). *J. Ass. Phycns India*, **10**, 225.
- Meyering, C. A., Israels, A. L. M., Sebens, T., and Huisman, T. H. J. (1960). Studies on the heterogeneity of hemoglobin. II. The heterogeneity of different human hemoglobin types in carboxymethylcellulose and in Amberlite IRC-50 chromatography: quantitative aspects. *Clin. chim. Acta*, **5**, 208.
- Minnich, V., Cordonnier, J. K., Williams, W. J., and Moore, C. V. (1962). Alpha, beta and gamma hemoglobin polypeptide chains during the neonatal period with description of a fetal form of hemoglobin D α St. Louis. *Blood*, **19**, 137.
- , Na-Nakorn, S., Tuchinda, S., Pravitt, W., and Moore, C. V. (1956). Inclusion body anemia in Thailand (Hemoglobin H-thalassaemia disease). *Proc. 6th Congr. Int. Soc. Haemat.*, 743.
- Miyaji, T., Iuchi, I., Shibata, S., Takeda, I., and Tamura, A. (1963a). Possible amino acid substitution in the α -chain (α 87 Tyr) of Hb Miwate. *Acta haemat. jap.*, **26**, 538.
- , Takeda, I., and Shibata, S. (1963b). Haemoglobin Shimonoesei (α ¹⁴Arg β ⁴), a slow-moving hemoglobin found in a Japanese family, with special reference to its chemistry. *ibid.*, **26**, 531.
- Moore, M. M., and Pearson, H. A. (1964). Human hemoglobin gene linkage with an apparent crossing over between thalassemia and delta loci. *Clin. Res.*, **12**, 34.
- Motulsky, A. G. (1962). Controller genes in synthesis of human haemoglobin. *Nature (Lond.)*, **194**, 607.
- (1964a). Hereditary red cell traits and malaria. *Amer. J. trop. Med. Hyg.*, **13**, 147.
- (1964b). Thalassaemia. *Cold Spr. Harb. Symp. quant. Biol.*, **29**, In the press.
- Muirhead, H., and Perutz, M. F. (1963). Structure of haemoglobin. A three-dimensional Fourier synthesis of reduced human haemoglobin at 5.5 Å resolution. *Nature (Lond.)*, **199**, 633.
- Muller, C. J. (1960). Separation of the α - and β -chains of globins by means of starch-gel electrophoresis. *ibid.*, **186**, 643.
- (1961). *A Comparative Study of the Structure of Mammalian and Avian Haemoglobins*, Doctoral Thesis.
- , and Jonxis, J. H. P. (1960). Identity of haemoglobin A₁. *Nature (Lond.)*, **188**, 949.
- , and Kingma, S. (1961). Haemoglobin Zurich α ₁ β ₂⁴³Arg. *Biochim. biophys. Acta (Amst.)*, **50**, 595.
- Murawski, K., Antonini, E., Brundri, M., Carta, S., Rossi-Fanelli, A., Sorcini, M., Tentori, L., Vivaldi, G., and Wyman, J. (1964). The functional and structural abnormality of hemoglobin M_{Radom}. *Proc. Int. Soc. Haemat.*, **10**, 1964.
- Murayama, M. (1962a). Chemical difference between normal human haemoglobin and haemoglobin-I. *Nature (Lond.)*, **196**, 276.
- (1962b). A sub-molecular mechanism of gel formation in sickle cell haemolysate. *ibid.*, **194**, 933.
- (1964). A molecular mechanism of sickled erythrocyte formation. *ibid.*, **202**, 258.
- Na-Nakorn, S., and Minnich, V. (1957). Studies on hemoglobin E. III. Homozygous hemoglobin E and variants of the lassemia and hemoglobin E. A family study. *Blood*, **12**, 529.
- Neeb, H., Beiboer, J. L., Jonxis, J. H. P., Kaars Sijpesteijn, J. A., and Muller, C. J. (1961). Homozygous Lepore haemoglobin disease appearing as thalassaemia major in two Papuan siblings. *Trop. geogr. Med.*, **13**, 207.
- Neel, J. V. (1949). The inheritance of sickle cell anemia. *Science*, **110**, 64.
- (1956). The genetics of human haemoglobin differences: problems and perspectives. *Ann. hum. Genet.*, **21**, 1.
- (1961). The hemoglobin genes: a remarkable example of the clustering of related genetic functions on a single mammalian chromosome. *Blood*, **18**, 769.
- Nirenberg, M. W., Jones, O. W., Leder, P., Clark, B. F. C., Sly, W. S., and Pestka, S. (1963). On the coding of genetic information. *Cold Spr. Harb. Symp. quant. Biol.*, **28**, 549.
- O'Gorman, P., Allsopp, K. M., Lehmann, H., and Sukumaran, P. K. (1963). Sickle-cell haemoglobin K disease. *Brit. med. J.*, **2**, 1381.
- Ohya, I. (1963). Abnormal hemoglobins in North Kyshu, Japan, with special reference to Hb-Kokura and Hb-Fukuoka. *Jap. J. hum. Genet.*, **8**, 23.
- Pauling, L., Itano, H. A., Singer, S. J., and Wells, I. C. (1949). Sickle-cell anemia, a molecular disease. *Science*, **110**, 543.
- Pearson, H. A., Gerald, P. S., and Diamond, L. K. (1959). Thalassaemia intermedia due to interaction of Lepore trait with thalassaemia trait. *Amer. J. Dis. Child.*, **97**, 464.
- , and Vertrees, K. M. (1961). Site of binding of chromium-51 to haemoglobin. *Nature (Lond.)*, **189**, 1019.
- Penrose, L. S., Smith, S. M., and Sprott, D. A. (1956). On the stability of allelic systems, with special reference to haemoglobins A, S, and C. *Ann. hum. Genet.*, **21**, 90.

- Perutz, M. F. (1964). Structure and function of haemoglobin. In Symposium: the structure and activity of enzymes. Proceedings of the 1st meeting of the Federation of European Biochemical Societies, p. 141.
- , Rossmann, M. G., Cullis, A. F., Muirhead, H., Will, G., and North, A. C. T. (1960). Structure of haemoglobin. A three-dimensional Fourier synthesis at 5.5 Å resolution, obtained by X-ray analysis. *Nature (Lond.)*, **185**, 416.
- Pierce, L. E., Rath, C. E., and McCoy, K. (1963). A new hemoglobin variant with sickling properties. *New Engl. J. Med.*, **268**, 862.
- Pisciotta, A. V., Ebbe, S. N., and Hinz, J. E. (1959). Clinical and laboratory features of two variants of methemoglobin M disease. *J. Lab. clin. Med.*, **54**, 73.
- Poulík, M. D. (1957). Starch gel electrophoresis in a discontinuous system of buffers. *Nature (Lond.)*, **180**, 1477.
- Powers, D., Rohde, R., and Graves, D. (1964). Foetal haemoglobin and neutrophil anomaly in the D₁-trisomy syndrome. *Lancet*, **1**, 1363.
- Pribilla, W. (1962). Thalassämie-ähnliche Erkrankung mit neuem Minor-Hämoglobin (Hb Köln). In *Haemoglobin Colloquium, Vienna 1961*, ed. H. Lehmann and K. Betke, p. 73. Thieme, Stuttgart.
- Pugh, R. P., Monical, T. V., and Minnich, V. (1964). Sick cell anemia with two adult hemoglobins—Hb S and Hb G^{Philadelphia}. *S. Blood*, **23**, 206.
- Ramot, B., Sheba, Ch., Fisher, S., Ager, J. A. M., and Lehmann, H. (1959). Haemoglobin H disease with persistent haemoglobin 'Bart's' in an Oriental Jewess and her daughter; a dual alpha-chain deficiency of human haemoglobin. *Brit. med. J.*, **2**, 1228.
- Ranney, H. M. (1954). Observations on the inheritance of sickle-cell hemoglobin and hemoglobin C. *J. clin. Invest.*, **33**, 1634.
- , Jacobs, A. S., Bradley, T. B., Jr., and Cordova, F. A. (1963). A 'new' variant of haemoglobin A₂ and its segregation in a family with haemoglobin S. *Nature (Lond.)*, **197**, 164.
- , O'Brien, C., and Jacobs, A. S. (1962). An abnormal human foetal haemoglobin with an abnormal alpha-polypeptide chain. *ibid.*, **194**, 743.
- Raper, A. B. (1956). Sickling in relation to morbidity from malaria and other diseases. *Brit. med. J.*, **1**, 965.
- , Gammack, D. B., Huehns, E. R., and Shooter, E. M. (1960). Four haemoglobins in one individual. A study of the genetic interaction of Hb-G and Hb-C. *ibid.*, **2**, 1257.
- Reichlin, M., Bucci, E., Antonini, E., Wyman, J., and Rossi-Fanelli, A. (1964). The immunochemical difference between horse oxy- and deoxyhaemoglobin. *J. molec. Biol.*, **9**, 785.
- Reissman, K. R., Ruth, W. E., and Nomura, T. (1961). A human hemoglobin with lowered oxygen affinity and impaired heme-heme interactions. *J. clin. Invest.*, **40**, 1826.
- Rigas, D. A., Koler, R. D., and Osgood, E. E. (1955). New hemoglobin possessing a higher electrophoretic mobility than normal adult hemoglobin. *Science*, **121**, 372.
- , —, and — (1956). Hemoglobin H: clinical, laboratory and genetic studies of a family with a previously undescribed hemoglobin. *J. Lab. clin. Med.*, **47**, 51.
- Rimington, C. (1959). Biosynthesis of haemoglobin. *Brit. med. Bull.*, **15**, 19.
- Robinson, A. R., Robson, M., Harrison, A. P., and Zuelzer, W. W. (1957). A new technique for differentiation of hemoglobin. *J. Lab. clin. Med.*, **50**, 745.
- Robinson, E. A., and Itano, H. A. (1960). Identification of the recombinant products of canine and human haemoglobins. *Nature (Lond.)*, **188**, 798.
- Rosa, J., and Labie, D. (1962). Change in A₃ haemoglobin due to β-chain. *ibid.*, **196**, 901.
- Rossi-Fanelli, A., Antonini, E., and Caputo, A. (1958). Pure native globin from haemoglobin, preparation and some physico-chemical properties. *Biochim. biophys. Acta (Amst.)*, **28**, 221.
- , —, and — (1964). Hemoglobin and myoglobin. *Advanc. Protein Chem.*, **19**, 74.
- Rucknagel, D. L., and Chernoff, A. I. (1955). Immunologic studies of hemoglobins. III. Fetal hemoglobin in the circulation of pregnant women. *Blood*, **10**, 1092.
- , and Neel, J. V. (1961). The hemoglobinopathies. In *Prog. med. Genet.*, **1**, 158.
- Sahawi, E., Hunger, H., and Betke, K. (1962). Sporadisches Auftreten von Hb-M (Boston type?) in einer mitteldeutschen Familie. *Schweiz. med. Wschr.*, **92**, 1090.
- Salomon, H., Tatarski, I., Dance, N., Huehns, E. R., and Shooter, E. M. (1964). A new hemoglobin variant found in a Bedouin tribe: hemoglobin 'Rambam'. Proc. of the International Congress of Pacific and Asiatic Pathology Soc. Haifa, 1964. In the press.
- Sarabhai, A. S., Stretton, A. O. W., Brenner, S., and Bolle, A. (1964). Co-linearity of the gene with the polypeptide chain. *Nature (Lond.)*, **201**, 13.
- Schmid, R., Brecher, G., and Clemens, T. (1959). Familial hemolytic anemia with erythrocyte inclusion bodies and a defect in pigment metabolism. *Blood*, **14**, 991.
- Schneider, R. G., and Arat, F. (1964). Immunological relationships of various types of haemoglobin. I. Reactions of antisera A, F and 'Bart's' to haemoglobins with various polypeptide chains. *Brit. J. Haemat.*, **10**, 15.
- , —, and Haggard, M. E. (1964a). An inhomogeneous foetal haemoglobin variant (Texas type). *Nature (Lond.)*, **202**, 1346.
- , Haggard, M. E., McNutt, C. W., Johnson, J. E., Jr., Bowman, B. H., and Barnett, D. R. (1964b). Haemoglobin G^{Coushatta}: a new variant in an American Indian family. *Science*, **143**, 697.
- Schnek, A. G., and Schroeder, W. A. (1961). The relation between the minor components of whole normal adult hemoglobin as isolated by chromatography and starch block electrophoresis. *J. Amer. chem. Soc.*, **83**, 1472.
- Schroeder, W. A. (1959). The chemical structure of normal human hemoglobins. *Fortschr. Chem. org. Natstoffe*, **17**, 322.
- (1963). The hemoglobins. *Ann. Rev. Biochem.*, **32**, 301.
- , Cua, J. T., Matsuda, G., and Fenninger, W. D. (1962). Hemoglobin F₁: an acetyl-containing hemoglobin. *Biochim. biophys. Acta (Amst.)*, **63**, 532.
- , and Matsuda, G. (1958). N-terminal residues of human fetal hemoglobin. *J. Amer. chem. Soc.*, **80**, 1521.
- , Shelton, J. R., Shelton, J. B., and Cormick, J. (1962). Further sequences in the γ-chain of human fetal hemoglobin. *Proc. Nat. Acad. Sci. (Wash.)*, **48**, 284.
- , Sturgeon, P., and Bergren, W. R. (1962). Chemical investigation of haemoglobin F from an individual with persistent foetal haemoglobin. *Nature (Lond.)*, **193**, 1161.
- Scott, E. M. (1960). The relation of diaphorase of human erythrocytes to inheritance of methemoglobinemia. *J. clin. Invest.*, **39**, 1176.
- Scott, J. L., Haut, A., Cartwright, G. E., and Wintrobe, M. M. (1960). Congenital hemolytic disease associated with red cell inclusion bodies, abnormal pigment metabolism and an electrophoretic hemoglobin abnormality. *Blood*, **16**, 1239.
- Shepard, M. K., Weatherall, D. J., and Conley, C. L. (1962). Semi-quantitative estimation of the distribution of fetal hemoglobin in red cell populations. *Bull. Johns Hopk. Hosp.*, **110**, 293.
- Sherman, I. J. (1940). The sickling phenomenon, with special reference to the differentiation of sickle cell anemia from the sickle cell trait. *ibid.*, **67**, 309.
- Shibata, S., and Iuchi, I. (1962). Agar gel electrophoresis for rapid separation of hemoglobin. *Proc. Int. Soc. Haemat., Tokyo*, 1960, **8** (2), 1239.
- , Miyaji, T., Ueda, S., and Takeda, I. (1963). Hemolytic disease associated with the production of abnormal hemoglobin and intraerythrocytic Heinz bodies. *Acta haemat. Jap.*, **26**, 164.
- , —, Ueda, S., Miyaji, T., and Takeda, I. (1962b). Agar gel electrophoresis of the hybrid of canine and human haemoglobins: a simple convenient means for the determination of chain anomaly. *ibid.*, **25**, 675.
- , Miyaji, T., Iuchi, I., Ueda, S., and Takeda, I. (1964). Hemoglobin Hikari (α₂β₂¹ASP¹HE²): a fast-moving hemoglobin found in two unrelated Japanese families. *Clin. chim. Acta*, **10**, 101.
- , —, —, —, Kimura, N., and Kodama, S. (1962a). Hemoglobin M-Kurume: its identity with hemoglobin M-Saskatoon. *Acta haemat. Jap.*, **25**, 690.
- Shooter, E. M., Skinner, E. R., Garlick, J. P., and Barnicot, N. A. (1960). The electrophoretic characterization of haemoglobin G and a new minor haemoglobin, G₁. *Brit. J. Haemat.*, **6**, 140.
- Silvestroni, E., and Bianco, I. (1958). Associazione di Hb G e microcitemia in due membri di una famiglia italiana del ferrarese. *Polliclinico, Sez. prat.*, **65**, 203.
- , and — (1963). A new variant of human fetal hemoglobin: Hb F_{roma}. *Blood*, **22**, 545.

- , —, and Brancati, C. (1963). Haemoglobins N and P in Italian families. *Nature (Lond.)*, **200**, 658.
- Singer, K., Chernoff, A. I., and Singer, L. (1951). Studies on abnormal hemoglobins. I. Their demonstration in sickle-cell anemia and other hematologic disorders by means of alkali denaturation. *Blood*, **6**, 413.
- , and Fisher, B. (1952). Studies on abnormal hemoglobins. V. The distribution of type S (sickle cell) hemoglobin and type F (alkali resistant) hemoglobin within the red cell population in sickle cell anemia. *ibid.*, **7**, 1216.
- , Kraus, A. P., Singer, L., Rubinstein, H. M., and Goldberg, S. R. (1954). Studies on abnormal hemoglobins. X. A new syndrome: hemoglobin C-thalassemia disease. *ibid.*, **9**, 1032.
- , Singer, L., and Goldberg, S. R. (1955). Studies on abnormal hemoglobins. XI. Sickle cell-thalassemia disease in the Negro. The significance of the S + A + F and S + A patterns obtained by hemoglobin analysis. *ibid.*, **10**, 405.
- Smith, E. W., and Torbert, J. V. (1958). Study of two abnormal hemoglobins with evidence for a new genetic locus for hemoglobin formation. *Bull. Johns Hopk. Hosp.*, **102**, 38.
- Spaet, T. H., Alway, R. H., and Ward, G. (1953). Homozygous type 'c' hemoglobin. *Pediatrics*, **12**, 483.
- Steiner, M., Baldini, M., and Dameshek, W. (1964). Studies of haem synthesis in thalassaemia, refractory sideroblastic anaemia and the di Guglielmo Syndrome. *Proc. int. Soc. Haemat.*, **10**. In the press.
- Stern, C. (1960). *Principles of Human Genetics*, 2nd ed. W. H. Freeman, San Francisco and London.
- Sturgeon, P., Schroeder, W. A., Jones, R. T., and Bergren, W. R. (1963). The relation of alkali-resistant haemoglobin in thalassaemia and abnormal haemoglobin syndromes to foetal haemoglobin. *Brit. J. Haemat.*, **9**, 438.
- Swenson, R. T., Hill, R. L., Lehmann, H., and Jim, R. T. S. (1962). A chemical abnormality in hemoglobin G from Chinese individuals. *J. biol. Chem.*, **237**, 1517.
- Teale, F. W. J. (1959). Cleavage of the haem-protein link by acid methylethylketone. *Biochim. biophys. Acta (Amst.)*, **35**, 543.
- Thompson, G. R., and Lehmann, H. (1962). Combinations of high levels of haemoglobin F with haemoglobins A, S, and C in Ghana. *Brit. med. J.*, **1**, 1521.
- Thompson, R. B., Mitchener, J. W., and Huisman, T. H. J. (1961). Studies on the fetal hemoglobin in the persistent high Hb-F anomaly. *Blood*, **18**, 267.
- Tondo, C. V., Salzano, F. M., and Rucknagel, D. L. (1963). Hemoglobin Porto Alegre, a possible polymer of normal hemoglobin in a Caucasian Brazilian family. *Amer. J. hum. Genet.*, **15**, 265.
- Townes, P. L., and Morrison, M. (1962). Investigation of the defect in a variant of hereditary methemoglobinemia. *Blood*, **19**, 60.
- Tuchinda, S., Rucknagel, D. L., Minnich, V., Boonyaparakob, U., Balankura, K., and Suvatee, V. (1964). The coexistence of genes of hemoglobin E and α -thalassaemia in Thais, with resultant suppression of hemoglobin E synthesis. *Amer. J. hum. Genet.*, **16**, 311.
- Vandepitte, J., and Delaisse, J. (1957). Sicklémie et paludisme. *Ann. Soc. belge Méd. trop.*, **37**, 703.
- Van Vliet, G., and Huisman, T. H. J. (1964). Changes in the hemoglobin types of sheep as a response to anaemia. *Biochem. J.*, **93**, 401.
- Vella, F., Ager, J. A. M., and Lehmann, H. (1959). New variant of human foetal haemoglobin. *Nature (Lond.)*, **183**, 31.
- , Wells, R. H. C., Ager, J. A. M., and Lehmann, H. (1958). A haemoglobinopathy involving haemoglobin H and a new (Q) haemoglobin. *Brit. med. J.*, **1**, 752.
- Vinograd, J., and Hutchinson, W. D. (1960). Carbon-14 labelled hybrids of haemoglobin. *Nature (Lond.)*, **187**, 216.
- Wasi, P., Na-Nakorn, S., and Suingdamrong, A. (1964). Thalassaemia-Hb-H in Thailand: a genetical study. *Proc. Xth Cong. int. Soc. Haemat.* In the press.
- Weatherall, D. J., and Baglioni, C. (1962). A fetal hemoglobin variant of unusual genetic interest. *Blood*, **20**, 675.
- , and Boyer, S. H., IV (1962). Evidence for the genetic identity of alpha chain determinants in hemoglobins A, A₁, and F. *Bull. Johns Hopk. Hosp.*, **110**, 8.
- , Sigler, A. T., and Baglioni, C. (1962). Four hemoglobins in each of three brothers. Genetic and biochemical significance. *ibid.*, **111**, 143.
- Went, L. N., and MacIver, J. E. (1958). An unusual type of hemoglobinopathy resembling sickle cell-thalassemia disease in a Jamaican family. *Blood*, **13**, 559.
- Westendorp Boerma, F., and Huisman, T. H. J. (1964). Serological investigations of human hemoglobins. II. Antibodies produced by isolated human hemoglobin types with known structural differences. *J. Lab. clin. Med.*, **63**, 264.
- , —, and Mandema, E. (1960). Serological investigations of some human and animal hemoglobins by agar gel-diffusion method. *Clin. chim. Acta*, **5**, 564.
- Wheeler, J. T., and Krevans, J. R. (1961). The homozygous state of persistent fetal hemoglobin and the interaction of persistent fetal hemoglobin with thalassemia. *Bull. Johns Hopk. Hosp.*, **109**, 217.
- White, J. C., and Beaven, G. H. (1959). Foetal haemoglobin. *Brit. med. Bull.*, **15**, 33.
- , Ellis, M., Coleman, P. N., Beaven, G. H., Gratzner, W. B., Shooter, E. M., and Skinner, E. R. (1960). An unstable haemoglobin associated with some cases of leukaemia. *Brit. J. Haemat.*, **6**, 171.
- Wilson, S., and Smith, D. B. (1959). Separation of the valyl-leucyl- and valyl-glutamyl-polypeptide chains of horse globin by fractional precipitation and column chromatography. *Canad. J. Biochem.*, **37**, 405.
- Winterhalter, K. H., and Huehns, E. R. (1963). Free globin in red cells. *J. clin. Invest.*, **42**, 995.
- , and — (1964). Preparations, properties and specific recombination of $\alpha\beta$ globin subunits. *J. biol. Chem.*, **239**, 3699.
- Wintrobe, M. M. (1961). *Clinical Hematology*, 5th ed. Kimpton London.
- Yakulis, V. J., Heller, P., Josephson, A. M., and Singer, L. (1960). Rapid demonstration of A₂ hemoglobin by means of agar gel electrophoresis. *Amer. J. clin. Path.*, **34**, 28.
- Yanofsky, C., Carlton, B. C., Guest, J. R., Helinski, D. R., and Henning, U. (1964). On the colinearity of gene structure and protein structure. *Proc. nat. Acad. Sci. (Wash.)*, **51**, 266.
- Zuckerklund, E. (1964). Controller-gene diseases. The operon model applied to β -thalassaemia, familial fetal hemoglobinemia and the normal switch from the production of fetal hemoglobin to that of adult hemoglobin. *J. molec. Biol.*, **8**, 128.
- Zuelzer, W. W., and Kaplan, E. (1954). Thalassaemia-hemoglobin C disease. *Blood*, **9**, 1047.
- , Robinson, A. R., and Booker, C. R. (1961). Reciprocal relationship of hemoglobins A₂ and F in beta chain thalassemias, a key to the genetic control of hemoglobin F. *ibid.*, **17**, 393.