PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 506th Meeting of the Society was held at the University of Cambridge on Tuesday and Wednesday, 7 and 8 July 1970, when the following papers were presented:

COLLOQUIUM ON 'HAEMOGLOBIN AND MYOGLOBIN'

Stereochemical Effects of Amino Acid Substitution in Abnormal Human Haemoglobin

By M. F. PERUTZ and J. GREER. (*Medical Research Council Laboratory of Molecular Biology Cambridge, CB2 2QH, U.K.*)

Over 100 mutant human haemoglobins are now known; in most of them a single pair of amino acid residues is replaced in either the α - or the β -chains. About half the replacements occur on the surface of the haemoglobin molecule and are innocuous, at least in heterozygotes. Replacements in one of the two pairs of contacts between α - and β -chains ($\alpha_1\beta_2$) tend to affect the interaction between the haem molecules, so that the oxygen-equilibrium curve becomes hyperbolic rather than sigmoid (Perutz & Lehmann, 1968; Perutz, 1969).

X-ray analyses of several of these mutant haemoglobins have been carried out by using a method that is very sensitive to small perturbations of the mutant as compared with the wild-type structure. The results show a great variety of effects, some predictable, others unexpected. Predictably, a replacement of an asparagine residue by a threonine residue in a position where the γ -methyl group of the threonine residue gets in the way of the porphyrin ring causes perturbations throughout the β -subunits, but unexpectedly these perturbations also extend to the α -subunits [haemoglobin Kansas, G4(102) β]. Replacement of an arginine residue by a leucine residue at FG4(92) α causes no perturbations in the deoxy form, but extensive ones in the oxy form, of the molecule (Greer, 1970).

The properties of these mutant haemoglobins suggest that the most important pathway of haem-haem interaction goes through the contact $\alpha_1\beta_2$. Haem-haem interaction cannot occur if the structural transition between the oxy and deoxy forms is inhibited. We had therefore expected such inhibition to occur in the mutant haemoglobins where Hill's constant is close to unity. However, this is not true. It appears that small perturbations in either the oxy or the deoxy forms may be sufficient to stop haem-haem interaction, for reasons which are not yet clear.

Perutz, M. F. (1969). *Proc. R. Soc. B*, **173**, 113.

Perutz, M. F. & Lehmann, H. (1968). *Nature, Lond.*, **219**, 902.

Greer, J. (1970). Ph.D. Dissertation: University of Cambridge.

Vertebrate and Insect Haemoglobins

By GERHARD BRAUNITZER. (*Max-Planck-Institut für Biochemie, Munich, Germany*)

Chironomus, a relative of *Drosophila*, possesses only four giant chromosomes. It has been reported that there are over 1500 species of *Chironomus* (Thienemann, 1950). I have studied the haemoglobins obtained from the larvae of *Chironomus thummi thummi* (Diptera). Large amounts of these genetically uniform larvae, known to contain about 1% of haemoglobin, could be obtained in Europe. Svedberg (1934) has shown that the molecular weight of haemoglobin isolated from *Chironomus plumosus* is in the order of 32000.

A great number of haemoglobins are present in *Chironomus thummi th.*, as shown by the analysis of haemoglobin components. These haemoglobins were separated into a monomer and a dimer form by Sephadex chromatography. However, as shown by column chromatography (Braun, Crichton & Braunitzer, 1968), they contain at least 12 different components, which differ from each other in a uniform and continuous manner. Most of these haemoglobins can be differentiated by N- and C-terminal end-group analysis. The amino acid ratios of these haemoglobins vary enormously.

Also, relative to haemoglobins isolated from other species, they contain few histidine residues and a great number of apolar residues, especially isoleucine and phenylalanine. I have crystallized one of the monomer forms, component III (CTT-III), and have recently determined its primary structure through the sequence analysis. The amino acid sequence of component CTT-III is variable in two positions; these positions contain two simultaneous substitutions of neutral amino acids (Thr/Ile and Pro/Ile). Further, two amino acid insertions, one leucine and one alanine, were

found in the primary structure of CTT-III. Therefore, this component can be shown to contain at least four different haemoglobins, the lengths of which vary from 135 to 137 amino acids. Its primary structure (Buse, Braig & Braunitzer, 1969) shows enormous differences when compared with the haemoglobins of vertebrates. The average mutation ratio between component CTT-III and the haemoglobin chain of humans is 1.22 mutations per codon. It has also been observed that there are now only three invariant amino acids.

The N-terminal amino acid sequence of component CTT-III is identical, except for one deletion, with those of the horse haemoglobins. The proximal histidine residue of vertebrate haemoglobin is present in position F8 in component CTT-III. However, the distal histidine residue, which is at position E7 in the vertebrate chains, could not be found. It is replaced by glutamic acid. The sixth co-ordination site of the haem group of component CTT-III shifts just one turn of the helix, from position E7 to position E11, as shown from X-ray results (Huber, 1969). Further, by chemical means isoleucine was found in position E11 instead of histidine. Therefore, there seems to be a replacement of the vertebrate distal histidine residue in the *Chironomus* haemoglobin.

Component CTT-III contains only one tryptophan residue, which is found in the C-terminal region. The amino acid sequences of this region (Ala-Ala-Trp-Gly-Thr-Leu) are identical or homologous (Braunitzer, Busse & Braig, 1969) with the tryptophan-containing sequences found in positions 10-14 in the haemoglobins of mammals and in myoglobins. This evidence suggests an intragene duplication. The hypothesis that the present haemoglobin genes are derived from the older, smaller, genes will be discussed. Preliminary results concerning the primary structure of *Chironomus* components CTT-I, CTT-IV and CTT-X will also be mentioned.

- Braun, V., Crichton, R. R. & Braunitzer, G. (1968). *Hoppe-Seyler's Z. physiol. Chem.* **349**, 197.
 Braunitzer, G., Buse, G. & Braig, S. (1969). *Hoppe-Seyler's Z. physiol. Chem.* **350**, 1477.
 Buse, G., Braig, S. & Braunitzer, G. (1969). *Hoppe-Seyler's Z. physiol. Chem.* **350**, 1686.
 Huber, R., Epp, O. & Formanek, H. (1969). *Naturwissenschaften*, **56**, 362.
 Svedberg, Th. & Eriksson-Quensel, I. B. (1934). *J. Am. chem. Soc.* **56**, 1700.
 Thienemann, A. (1950). *Chironomus*. Stuttgart: E. Schweizerbarthsche Verlagsbuchhandlung.

Mixed Haemoglobin Molecules

By G. A. GILBERT. (*Department of Biochemistry, University of Birmingham, Birmingham 15, U.K.*)

According to the evidence provided by gel electrophoresis, hybrid haemoglobin molecules of the general type $(\alpha^x\beta^y)_2$ exist *in vivo* (see Lehmann & Huntsman, 1966) and can be formed *in vitro* by exposing mixtures of $(\alpha^x\beta^x)_2$ and $(\alpha^y\beta^y)_2$ temporarily to acid or alkaline conditions (Singer & Itano, 1959; Vinograd & Hutchinson, 1960; see also Huehns & Shooter, 1965). In complete contrast, hybrids of the type $(\alpha^x\beta^x, \alpha^y\beta^y)$ are never, or almost never, to be found by gel electrophoresis, and until recently it was widely believed that such hybrids are energetically unstable. Guidotti, Konigsberg & Craig (1963) perceived that methods of analysis such as electrophoresis would tend to dissociate such hybrids, leaving only the symmetrical tetramers $(\alpha^x\beta^x)_2$ and $(\alpha^y\beta^y)_2$ to be detected. Subsequently Guidotti (1967) confirmed, by osmotic-pressure measurements carried out on mixtures of modified haemoglobins under partially dissociating conditions, that hybrids of the asymmetrical type were actually present, confirming by implication that the rapidly reversible nature of the dissociation of haemoglobin hybrid tetramers into dimers had prevented their detection by gel electrophoresis.

Free-boundary electrophoresis also has been widely thought to show that asymmetrical tetramers are absent in such natural mixtures of haemoglobins as occur, for example, in sickle-cell trait (Pauling, Itano, Singer & Wells, 1949), because two instead of an expected three peaks are displayed by the boundaries. For similar reasons, β -lactoglobulin was supposed not to form hybrids when the A and B forms were mixed (Townend, Kiddy & Timasheff, 1961). However, this is not a necessary deduction from such experiments, and in fact moving-boundary theory predicts only two peaks, one of these peaks being a reaction peak not representative of any of the individual species. Many of the published electrophoretic patterns of haemoglobin mixtures, and those of the mixed β -lactoglobulins, show the theoretically expected area and velocity adjustments, which indicate the presence of appreciable amounts of hybrids. Indeed these patterns are susceptible to analysis to determine what proportion of haemoglobin is present as a hybrid.

A mixture of two haemoglobins may be represented formally by the reversible reaction

By using this scheme, the shape of the asymptotic, i.e. 'diffusion-free' boundaries to be expected on electrophoresis can be calculated by the method of