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