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

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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

Bethune & Kegeles (1961a,b,c), as shown by Hill (1964), or by numerical integration (Gilbert & Gilbert, 1965) of the conservation-of-mass equations (Gilbert & Jenkins, 1959). Two peaks, not three, are found by such calculation in the descending and ascending boundaries in free-boundary electrophoresis, in agreement with the experimental results.

It is desirable to determine as exactly as possible the relative stability of the hybrid compared with the pure components. It seems possible that this may be achieved by various differential methods that allow a direct comparison of degrees of dissociation. One particular method has been tested by simulation calculations by Mrs L. M. Gilbert. In this method, in the nomenclature above, mixtures of A₂ and B₂ are layered (Gilbert, 1966) in a gel column over an exactly equal concentration of A₂ and under an exactly equal concentration of B₂. The experiment is repeated over a wide range of total concentrations, and from the difference in elution volumes calculated from observations at the junction of the solutions as they emerge from the columns, the required interaction energies can be calculated. The method has been successfully tested by Dr C. M. Page, using single-beam spectrophotometry, and is under further development by Miss N. Shawky, using a double-beam spectrophotometer to compare plateau and junction extinctions of the emerging solutions.

- Bethune, J. L. & Kegeles, G. (1961a). *J. phys. Chem., Ithaca*, **65**, 433.
 Bethune, J. L. & Kegeles, G. (1961b). *J. phys. Chem., Ithaca*, **65**, 1755.
 Bethune, J. L. & Kegeles, G. (1961c). *J. phys. Chem., Ithaca*, **65**, 1761.
 Gilbert, G. A. & Jenkins, R. C. Ll. (1959). *Proc. R. Soc. A*, **253**, 420.
 Gilbert, L. M. & Gilbert, G. A. (1965). *Biochem. J.* **97**, 7c.
 Gilbert, G. A. (1966). *Nature, Lond.*, **212**, 296.
 Guidotti, G. (1967). *J. biol. Chem.* **242**, 3694.
 Guidotti, G., Konigsberg, W. & Craig, L. C. (1963). *Proc. natn. Acad. Sci. U.S.A.* **50**, 774.
 Hill, R. J. (1964). *Brookhaven Symp. Biol.* **17**, 201.
 Huehns, E. R. & Shooter, E. M. (1965). *J. med. Genet.* **2**, 48.
 Lehmann, H. & Huntsman, R. G. (1966). *Man's Haemoglobins*. Amsterdam: North Holland Publishing Co.
 Pauling, L., Itano, H. A., Singer, S. & Wells, I. C. (1949). *Science, N.Y.*, **110**, 543.
 Singer, S. J. & Itano, H. A. (1959). *Proc. natn. Acad. Sci. U.S.A.* **45**, 174.
 Townend, R., Kiddy, C. A. & Timasheff, S. N. (1961). *J. Am. chem. Soc.* **83**, 1419.
 Vinograd, J. & Hutchinson, W. D. (1960). *Nature, Lond.*, **187**, 216.

Myoglobin, with Particular Reference to the Myoglobin of *Aplysia*

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Myoglobins, sometimes also called tissue haemoglobins, are respiratory pigments present in the muscles of all vertebrates and occasionally in those of invertebrates. They are composed of a protein molecule and a prosthetic group: the iron protoporphyrin IX, called haem. The haem can be considered to be the active group that gives to myoglobin the capacity of reversibly binding molecular oxygen.

This protein, which gives the red colour to the fresh muscle, is responsible for the storage and transportation of oxygen needed by the muscle cell. In fact, the content of myoglobin is particularly high in muscles characterized by repetitive activity and by long contraction times, e.g. the heart of large mammals and the leg muscles of running animals; it is also abundant in muscles of diving animals.

Myoglobins have higher oxygen affinity than that of haemoglobins and vascular respiratory pigments of vertebrates; on the other hand, as they contain a single haem group per molecule, their oxygen dissociation curve is hyperbolic instead of sigmoid, and they do not generally show a Bohr effect.

Sperm-whale myoglobin was the first myoglobin of which the three-dimensional structure was elucidated, in parallel with the complete amino acid sequence (Kendrew, 1963; Kendrew *et al.*, 1960; Edmundson, 1965). The polypeptide chain is twisted in a complex and asymmetric way, in order to form a flattened triangular prism of the following dimensions: 45 Å × 35 Å × 25 Å. The molecule is very compact and contains only five water molecules trapped inside the protein. The haem group is located almost normally to the surface of the molecule; the fifth co-ordination position of iron is occupied by the imidazole nitrogen of the proximal histidine residue. The sixth position of the iron atom, when the iron is in the ferric state, bonds to a molecule of water which in turn is bound by a hydrogen bond to the imidazole nitrogen of the distal histidine residue.

Myoglobins generally demonstrate the phenomenon of microheterogeneity both in vertebrates and in invertebrates (Satterlee, 1968); their polypeptide chain is formed of approx. 150 amino acid residues, with mol.wt. approx. 17000; in solution myoglobins are present in the form of monomers, the only exceptions being myoglobins of some molluscs. At the present time the primary structure of some vertebrate myoglobins is known (Genov, Shopova & Karadzova, 1968; Nedkov, Genov,