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.

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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,

Karadzova & Kreil, 1968; Bradshaw & Gurd, 1969; Dautrevaux, Boulanger, Han & Biserte, 1969), but information on the structure of myoglobins of invertebrates is scarce. The presence of myoglobins had been proved in the radular and buccal muscles, in the stomach, in the cartilage, in the heart and in the adductor muscles of many molluscs of the classes Amphineura, Gastropoda, Scaphopoda and Bivalva. Rossi-Fanelli and co-workers (Rossi-Fanelli & Antonini, 1957; Rossi-Fanelli, Antonini & Povoledo, 1958; Rossi-Fanelli, Antonini & Caputo, 1964) and Manwell (1964) and Read and co-workers (Read, 1966, 1967, 1968a,b; Koppenheffer & Read, 1968, 1969; Terwilliger & Read, 1969a,b) and Bannister and co-workers (Bannister, Bannister & Micallef, 1968) have reported partial results on the chemical structure and functional properties of some of these myoglobins. From the functional point of view the myoglobins of molluscs have the same significance as those of vertebrates: in fact they have a higher oxygen affinity than those of the respective vascular respiratory pigments and they are present in a higher quantity in molluscs involved in sustained rhythmic contractions. A phenomenon common to myoglobins of both molluscs and vertebrates is that of microheterogeneity. It varies with respect to the species of mollusc, both in the number of the minor components and in their quantity with respect to the main fraction. Also the molecular weight and the number of residues present in the polypeptide chain correspond fairly well to those of myoglobins of vertebrates. A peculiar characteristic of mollusc myoglobin is the presence of dimeric condition generally coexisting with the monomeric form.

Rossi-Fanelli and co-workers (Rossi-Fanelli & Antonini, 1957; Rossi-Fanelli *et al.* 1958) described the chemical and biochemical properties of the myoglobin of the opisthobranch gastropod *Aplysia limacina*. The findings of these authors have revealed a noteworthy property of *Aplysia* myoglobin: the polypeptide chain contains only one histidine residue. This is particularly notable in view of the role attributed to the iron-binding proximal and distal imidazole groups in mammalian haemoglobin and myoglobin. New results were reported (Tentori, Vivaldi, Carta, Antonini & Brunori, 1968) on the amino acid composition and peptide 'maps' of *Aplysia* myoglobin; these results have confirmed the presence of a single histidine residue and the absence of cysteine in the molecule. Noteworthy are also the absence of tyrosine and the presence of an acetylated N-terminal residue (Tentori, L., Vivaldi, C., Carta, S., Marinucci, M., Antonini, E. & Brunori, M., unpublished work).

Next the study of the sequence of amino acids of the polypeptide chain of *Aplysia limacina* myo-

globin was initiated. The results for the partial sequence of the last 63 residues at the C-terminal end of the molecule so far elucidated demonstrate that no similarity exists with the known sequences of mammalian myoglobins, at least for that of the C-terminal part of the molecule.

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Factors Affecting Intracellular Oxygen Affinity

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