HAEMOGLOBIN

Down Among the Dimers

from our Molecular Biology Correspondent

Two steps forward to one step back is perhaps the best rate of advance that can be hoped for in a complex field. Following the recent work by Kellett on the dissociation of liganded and unliganded haemoglobin, which I discussed a few weeks ago, the situation—the dust of conflict having apparently settled—appeared to be as follows: oxyhaemoglobin exists under normal conditions of salt and pH in an equilibrium state between tetramers and dimers, the concentration of the latter becoming significant only at extremely low protein concentrations. There are no monomers. Deoxygenated haemoglobin is tetrameric and does not dissociate to dimers even at the lowest measurable concentrations and at high ionic strength. What has also long been accepted, and successively confirmed by a series of workers, is that oxyhaemoglobin (or carboxyhaemoglobin and other liganded forms), even at high protein concentrations, dissociates in media of high ionic strength to dimers. Norén, Ho and Casassa (*Biochemistry*, **10**, 3222; 1971), however, say not so, and are prepared to take on the world.

The method which they use is light scattering with a laser source, the long wavelength of emission averting the difficulties caused by haem absorption. The results show that down to a concentration of less than 10^{-4} M in haem, 2 M sodium chloride has essentially no effect on the molecular weight. From the invariance of molecular weight with protein concentration over the measured range it follows that the tetramer-dimer equilibrium constant must certainly be lower than about 10^{-6} mole/l. Norén *et al.* then take a hatchet to earlier work, based variously on light scattering, osmometry, sedimentation equilibrium and sedimentation velocity. Earlier light scattering results, for example, stand condemned by internally inconsistent refractive index increment measurements, which failed to reveal the considerable dependence of this parameter on salt concentration. Other evidence is likewise revealed as flawed. The moral again is that work on nonideal polydisperse systems in mixed solvents is fraught with hazards.

The notion that lamprey haemoglobin, being apparently a monomer, would present a simpler system for study has also turned out to be risible. The oxygenation properties, it was soon established, are concentration dependent, and, worse yet, there is a Bohr effect. Briehl then found that the deoxygenated form is not predominantly monomeric. A re-examination of this

haemoglobin is now to be found in an article by Andersen (*J. Biol. Chem.*, **246**, 4800; 1971).

In the deoxyhaemoglobin the sedimentation equilibrium distributions can be fitted by equilibrium constants for association of monomers to dimers, and dimers to tetramers. The first process is pH-dependent and seems to involve a single charged group on each monomer. This at once explains the Bohr effect. The liganded haemoglobin also forms dimers at sufficiently high concentration, and apparently ultimately tetramers as well. At the haemoglobin concentration of 25 per cent in the red cell, the equilibrium constants as determined dictate that the deoxygenated pigment will be largely dimeric and the oxygenated form monomeric. Oxygenation will thus lead to dissociation of the protein. This will generate a measure of cooperativity at these physiological protein concentrations—a mechanism which may be seen as a precursor in this evolutionary prototype to that in more advanced models. In an accompanying article, Andersen and Gibson (*ibid.*, 4790) examine the kinetics of ligand binding by lamprey haemoglobin, and show that they can now be more or less quantitatively explained, assuming only that the monomer has a high and the dimer a low ligand affinity.

The cause of the earlier, and erroneous, impression that human oxyhaemoglobin would dissociate at sufficiently low concentrations to the monomeric state proved to be the ease with

which, under these conditions, the iron is oxidized to the ferric state. The methaemoglobin so formed has always been recognized as being unstable.

Bucci and Fronticelli (*Biochim. Biophys. Acta*, **243**, 170 ; 1971) have now examined the behaviour of isolated α-chains in the ferric state. The monomers are prepared by treatment of native haemoglobin with a thiol reagent, which can be stripped off after fractionation of the two chains. When the isolated chains were converted into the ferric state, the result was not in fact methaemoglobin, but a type with the characteristic absorption spectrum of a ferric haemochromogen, in which it is supposed that the iron is linked on both sides of the haem plane to a nitrogenous base. Judged by optical activity there is some loss of ordered structure, rather on the lines found in haem-free globin. In the ferric β-chains the effects are even more drastic. Reduction of the new type to the ferrous state failed to regenerate the native structure. The ferric chains evidently exist only in this modified and monomeric condition.

ADENOVIRUS

Hybridizers Beware

from our Cell Biology Correspondent

ANYBODY who has recourse to DNA. RNA hybridization techniques, and in particular competition hybridization, would be well advised to cast more than

Mouse Haemoglobin made *in vitro*

IN *Nature New Biology* next Wednesday two groups, Lockard and Lingrel and Mathews, Osborn and Lingrel, describe the synthesis *in vitro* of mouse globin and haemoglobin and conclude that the 9S murine globin messenger RNAs can be translated in cell free systems derived from ascites cells or reticulocytes of species other than the mouse. At least for this messenger neither tissue specific nor species specific initiation factors seem to be required for translation.

Lockard and Lingrel describe the translation of mouse reticulocyte 9S messenger RNA in a rabbit reticulocyte cell-free system and also mention that this messenger is translated in guinea-pig and duck reticulocyte systems. By fingerprinting the product of translation and by studying its behaviour on chromatography and gel filtration they conclude that both mouse α and β globin chains are being made and therefore the 9S RNA must contain messengers for both these molecules.

Mathews, Osborn and Lingrel have used this 9S RNA to programme a cell-free system obtained from mouse Krebs ascites cells, which were originally

derived from a mouse mammary carcinoma. They find that without the addition of any extract from reticulocytes both α and β globin chains are made. In short, the Krebs ascites cells contain all the factors required for the accurate initiation, elongation and termination of globin chains.

Recently Stavnezer and Huang reported in *Nature* (**230**, 172 ; 1971) that mouse messenger RNAs specifying the light chain of immunoglobulin are translated in the rabbit reticulocyte cell-free system *à la* Lingrel and Lockard. This result, taken together with those published in next Wednesday's *Nature New Biology*, suggests that the hypothesis that the translation of particular messengers depends on the existence of tissue-specification initiation factors may not rigorously apply to eukaryotic cells. Alternatively it can be suggested that tumour cells such as Krebs ascites cells, because they are cancerous, retain the capacity to translate a wide range of messengers and, further, that the factors in reticulocytes which initiate translation of globin messengers work also with immunoglobulin messengers.