MEMBRANES

Protein Patterns

from our Molecular Biology Correspondent

MOST of what biochemists learn at their mother's knee relates to the behaviour of biologically active molecules in aqueous solution. When confronted therefore with materials that are insoluble in water, and that reside and function in a condensed non-aqueous phase, they are apt to react with a baffled resentment, which explains perhaps the faltering touch so evident in much published work on the proteins of membranes. So far as red cell membranes are concerned, it is now apparent that the greatest cause of confusion has been the failure of relatively drastic treatments to achieve complete disaggregation, with consequent appearance of various associated forms in fractionation profiles. Lenard (*Biochemistry*, **9**, 5037; 1970) has taken advantage of the hard-won knowledge, that treatment with detergent at 100° C leads apparently to complete disaggregation, to compare the proteins from the red cells of five mammalian species, including man. It is now clear that the protein patterns in gel electrophoresis are in fact very similar, and that there are eight principal protein fractions in all cases, the largest of which have molecular weights of about 250,000. The pattern of labelling with a radioactive thiol reagent is also the same in all cases, with 50 per cent of the label in the high-molecular weight components. One prominent component in each membrane is a glycoprotein.

The search for the loci of specific activities in membranes has been assisted by the design of affinity labels. Kiefer *et al.* (*Proc. US Nat. Acad. Sci.*, **67**, 1688; 1970) have performed an affinity-labelling exercise on the acetylcholinesterase of red cells, as well as the acetylcholine receptor site of the neuromuscular junction in intact muscle. They have used a refinement of the method, whereby the reactivity of the label is triggered photochemically. The quaternary ammonium analogue of acetylcholine is attached to an aromatic azide. This can be photolysed to yield a highly reactive excited nitrene, which is expected to react with a variety of side chains. The advantages in this development are considerable: in the first place the binding properties of the ligand can be measured before irradiation. Second, non-specific reaction at points remote from the ligand site can be almost completely eliminated, because of the rapid decay of the excited species, assisted if need be by added scavengers in the buffer. It is only then the bound ligand that reacts with the protein within the lifetime of the active state. Kiefer *et al.* suggest that it may be possible to localize active sites by irradiation of a tissue in the presence of a label with a fine laser beam.

An altogether simpler system than the red cell, in terms at least of the multiplicity of constituents, is the retinal rod outer segment, which contains a stack of flat membrane bags, containing rhodopsin as effectively the sole, or at least predominant, protein. It therefore requires no very daring flight of the imagination to conjecture that the bleaching of the rhodopsin, which is already known to be accompanied by some kind of conformational adjustment, causes excitation by way of a change in the membrane permeability and thus a flux of ions. Now Heller, Ostwald and Bok (*Biochemistry*, **9**, 4884; 1970) have demonstrated that some such effect can indeed occur. The membranes, it is reported, are not permeable to alkali and alkaline earth ions, and when the osmotic pressure of the surrounding medium is changed they pick up or lose water to maintain osmotic balance. By recovering the particles from a suspension and weighing them, any volume changes can then be determined. Heller *et al.* find that whereas in the dark the volume remains almost constant, illumination in the presence of ATP and of sodium, potassium and calcium chlorides results in slow volume change of some 15 per cent, or 25 per cent of the interstitial space within the membranes. The membranes still maintain an ionic balance, however, which indicates that this is not simply a matter of leakage. Calcium chloride and ATP are both involved in mediating the change.

Though it cannot be denied that this is an interesting result, it must be recognized that its relation to the visual excitation process is not yet obvious: in the first place the volume change is slow, and furthermore it occurs at 4° C but not at 30° C. The suggestion of Heller *et al.* is that a change in membrane structure causes a change in passive transport of sodium and potassium against which the ATP-dependent pump has to work. The change is manifested only at 4° C when the pump velocity is diminished. This, however, is not easy to reconcile with the enhancing effect of ATP on the volume change.

MARINE BIOLOGY

Tuna, Starfish and Kelp

from a Correspondent

THE chief theme of the Challenger Society's meeting at the British Museum (Natural History) on January 13 and 14 was on the development of fisheries especially in African countries. The Challenger Society exists to promote the study of oceanography, but from time to time it has taken an interest in the ecology and fishery problems of the great lakes of Africa. The special lecture by Dr D. N. F. Hall (Overseas Development Administration, Foreign and Commonwealth Office) on the British aid programme to tropica

Control of Haemoglobin Synthesis

LITTLE is known about how the amount of protein synthesized by an active gene is controlled in higher organisms. The synthesis of haemoglobin is a particularly good system to use for investigating this problem, because haemoglobin is a direct gene product which is made in considerable quantity by reticulocyte cells, which can be readily obtained. Two studies on haemoglobin synthesis by Esan *et al.* and Nute and Stamatoyannopoulis, which will be published in next Wednesday's issue of *Nature New Biology*, were directed to this end.

Esan's group used human subjects who are heterozygous for the beta chain of haemoglobin. Such individuals must possess one gene which codes for a normal beta chain and one copy of its allele representing the mutant form. But the haemoglobin which they synthesize contains more of the normal than of the mutant type chain.

One explanation for this discrimination would be that wild type chains might be preferentially synthesized, either because mutant mRNA is unstable, or because it is translated more slowly or less frequently. Because reticulocytes possess no DNA and therefore cannot synthesize RNA, following protein synthesis shows directly what is happening to the translation of mRNA. When Esan *et al.* incubated reticulocytes with radioactive amino-acids, the haemoglobin produced followed the usual overall chain proportions; if differential production of protein were responsible for the mutation, the abnormal ratios of mutant to wild type chains would have been exaggerated. Esan *et al.* suggest therefore that the explanation for the selection may be that the alpha chain of haemoglobin prefers to associate with a normal beta chain rather than an aberrant one.

Human γ-haemoglobin is of more than one type (Schroeder *et al.*, *Proc. US Nat. Acad. Sci.*, **60**, 537; 1968), and each type seems to be coded by a separate gene. Nute and Stamatoyannopoulis have followed up this finding by looking at the haemoglobin of Macaque monkeys with electrophoresis on urea gels and analysis of peptide digests. They show that these monkeys seem to have at least two loci which code for the γ-haemoglobin polypeptide chain; this implies that one mechanism which cells may use to control the quantitative synthesis of proteins is the utilization of more than one gene to code for it. But it seems likely at present that this sort of mechanism is restricted to systems such as haemoglobin synthesis.