Internal cross-links were introduced in the tetramers of a haemoglobin A and S mixture with a bifunctional fluorodinitrobenzene, specifically *p,p'*-difluoro-*m,m'*-dinitrodiphenylsulphone. Gel electrophoresis after reaction revealed a third component, running between the A and S zones. This is not, of course, proof of the presence of a hybrid, for the annihilation of amino groups—actually as shown in earlier work, the α-chain termini — by the cross-linking reagent renders any identification based solely on electrophoretic mobility uncertain. Macleod and Hill therefore went on to isolate the hybrid in the following way: concentrated magnesium chloride was added to the mixture after reaction, so as to dissociate all dissociable haemoglobins into $\alpha\beta$ dimers. Any molecules cross-linked across their α or their β chains are unable to dissociate, and this fraction, which seems to be rather more than half of the total in the conditions used, is collected by gel filtration. Ion-exchange chromatography of this material gives rise to three principal fractions. By contrast the undissociable fraction in haemoglobins A and S mixed after separate exposure to the cross-linking agent generates only two components, corresponding to the first and the third species eluting from the heterologous system. The inference is that the middle peak is the cross-linked hybrid, $\alpha_2^A\beta^A\beta^S$, and it is shown to be such by analysis of the product of trypsin hydrolysis, in which the characteristic β^A and β^S peptides are present in equal amount.

The concentration of the cross-linked hybrid formed in an equimolar mixture of haemoglobins A and S is actually considerably greater than that of either of the homologous hybrids, and it therefore seems that the hybrid tetramer is not merely a significant, but in fact the preponderant, component in sickle-cell trait haemoglobin at equilibrium. This gives substance to a suggestion concerning the low probability of sickling in erythrocytes of sickle-cell heterozygotes, compared with homozygotes. It has been conjectured that the polymerization of the haemoglobin S in the cell is impeded by the incorporation of hybrid tetramers into the aggregates.

A different use for bifunctional reagents is as a means of restricting structural adjustments between the haemoglobin subunits during the oxygenation process. Cross-links within the β chains have been found sufficient to inhibit the transition to the low-affinity state. An interesting new essay in this direction comes from Fasold, Meyer and Steinkopff (*Europ. J. Biochem.*, **32**, 63 ; 1973), who have used a reagent based on iodoacetamide, with the built-in advantage that it can be cleaved in the middle by reduction of an azo link. The reagent is *p*-bis-iodoacetamide-2,2'-dicarboxyazobenzene; it is rigid, and can therefore only join reactive groups separated by a prescribed distance with rather little tolerance. Rapid reaction occurs with haemoglobin, with uptake of two moles of reagent per tetramer, after which a slower reaction supervenes.

The product of the first reaction, when fractionated on an ion-exchange column, contains one major component, which is tetramic, and possesses two α and two β chains, with one cross-link per β chain, for in a hydrolysate two groups in either are found to be carboxymethylated. The cross-link, which joins cys-93 to lys-82, is submerged in the molecule, more or less under the F helix. A minor product of the reaction also contains two cross-links, which, however, are asymmetrically disposed, one lying within a β chain, as in the major product, the other linking the cys-93 of the second β chain to his-45 of the neighbouring α chain. The corresponding monofunctional reagent blocked only the cys-93 of each β chain. The introduction of this foreign body into the vitals of the haemoglobin molecule caused a diminution in the haem-haem interaction, with a drop in the Hill constant to 1.7. With cross-links in the β chains, on the other hand, the Hill constant dropped to unity, and the same was found for the asymmetrically reacted species, in which only one β chain contains a cross-link. The authors suggest that the transition between the high and low-affinity conformational states may be repressed with some degree of independence at

DNA Synthesis in Developing Sea Urchins

In next Wednesday's *Nature New Biology* (March 7), Infante and his colleagues report the isolation from the nuclei of developing sea urchin embryos of a DNA-membrane complex which supports DNA synthesis both *in vivo* and *in vitro*. The natural synchrony of the first few cellular divisions after fertilization of the sea urchin egg facilitates study of the different stages of the division cycle.

Nuclei were isolated from embryos containing completely labelled DNA. The nuclei were lysed with detergent and the lysate was centrifuged on a discontinuous 15–40 per cent sucrose gradient. The bulk of the DNA was found free at the top of the gradient, but 10–30 per cent formed a band (the M-band) at the 40 per cent sucrose layer and was associated with membrane material. Treatment of the M-band complex with DNase, RNase, pronase, phospholipase-C and deoxycholate indicated that DNA, RNA, protein and phospholipids were all important in maintaining the integrity of the M-band.

When embryos in S-phase were pulse labelled with ^{3}H-thymidine for 30 s at 17° C, more than 70 per cent of the labelled DNA was located in the M-band. The remaining labelled DNA, located in the top fraction of the gradient, was demonstrated to be released DNA originating in the M-band when the labelling experiment was repeated at 5° C, at which temperature DNA synthesis is greatly retarded. At 5° C, all the DNA synthesized in a 30 s pulse was localized in the M-band. Although, following an extended labelling period of 10 min, the bulk of the DNA was found in the top fraction of the gradient, all of the DNA made in a 330 s pulse at the end of this 10 min period was located in the M-band. Thus DNA replication seems to occur at the membrane and non-replicating DNA is located in a fraction which is more readily dissociated from the membrane complex.

Further experiments, in which embryos were labelled for 35 min starting in the S-phase and ending in G2, showed that, as the cells entered G2, virtually no DNA could be found associated with the M-band. Thus the formation of the DNA-membrane complex may be a prerequisite for DNA synthesis. Furthermore, no M-band fraction could be found in mature unfertilized eggs—cells in which there is no DNA synthesis. When unfertilized eggs were mixed with embryos which had received a 30 s pulse during S-phase, an M-band fraction was obtained, showing that the absence of M-bands in unfertilized eggs was real. Thus the M-band seems to be formed soon after fertilization when DNA synthesis is initiated and disappears in G2 when synthesis is complete.

The M-bands and top fractions were isolated from early blastula nuclei and tested for their capacity to support DNA synthesis *in vitro*. No activity could be detected in the top fractions but the M-band fraction supported the synthesis of DNA which was dependent on the presence of all four deoxyribonucleotides.

These experiments must be interpreted with care. As suggested recently by Huberman *et al.* (*Nature*, **241**, 32 ; 1973) and Fakan *et al.* (*Proc. US Nat. Acad. Sci.*, **69**, 2300 ; 1972) during cell fractionation experiments it is possible that newly replicated DNA may possess special features such as single strandedness which may cause it to bind more protein or membrane material than bulk DNA. In other words, the M-band may be an artefact of cell lysis.