recently carried out in Hayflick's laboratory by Brautbar and his colleagues (*Exp. Cell Res.*, **75**, 31 ; 1972) supports the idea that there is no loss of HLA antigens in ageing diploid cells, for with cultures of WI38, WI26 and MRC-5 the same characteristic "tissue-type" was found even during the final senescent stages.

By contrast, Goldstein and Singal (*ibid.*, 278) report that in the strains of diploid fibroblasts which they have examined, loss of HLA antigens does occur and, as Sasportes found, this loss is detectable before the degeneration of a culture. In fact, it can even be used to diagnose the impending senescence of a culture.

The discrepancy between these various reports may have some purely technical explanation, the most likely one being that Brautbar and his colleagues examined only mass cultures of cells (Goldstein set out to look at cell clones rather than mass cultures). Indeed, because the mass cultures from which clones were derived showed no loss of HLA antigens it can be suggested that changes of antigen may be obscured when cells are grown *en masse*—probably as a result of the cooperation which is known to occur between cells in crowded conditions.

The method of assessing HLA may also have something to do with the discrepancy, for although Sasportes and Goldstein both used a dye exclusion method, Brautbar adopted a fluorochromatic one. Moreover, it is possible that the method of trypsinization may affect the result, for it is well known that the enzyme removes antigens from the surface of cells and it is therefore possible that the time at which HLA antigens are assessed after treatment is critical.

But can one interpret three negative reports and two positive ones in favour of the positive? The evidence which Goldstein provides with cell clones strongly suggests that one can, and it is interesting that with the different clones derived from a single cell strain the same HLA antigen is not always lost. Possibly this will support ideas which suppose that the molecular basis of ageing is of a random nature.

More important, however, is the fact that both Sasportes and Goldstein find that the loss of antigens is predominantly from the second of the HLA loci, the locus which is generally considered to be of greatest importance in so far as cell recognition is concerned. Might these results not therefore turn out to be a profoundly important link in what is known about ageing? If the loss of HLA antigens on these diploid fibroblasts reflects loss of antigens and surface membrane changes *in vivo* then it has important implications. Changes affecting either lymphoid or non-lymphoid cells would result in problems of self-recognition and loss of immunosurveillance so that autoimmune or neoplastic disease might follow.

True, it has yet to be established beyond any doubt that loss of HLA antigens or surface membrane changes do occur, but it is most attractive to interpret Sasportes's results and these recent results of Goldstein's in terms of immunological theories of ageing.

MEMBRANES

Lipophilia

from our Molecular Biology Correspondent

CURRENT beliefs about the state of proteins in membranes are encapsulated in artistic representations chiefly to be found in the pages of conference reports, in which they appear rather like marshmallows floating in a sea of treacle. The evidence is that, depending on the composition of the membrane, they can drift around with greater or lesser freedom, but that their orientation relative to the bilayer plane remains essentially invariant. There is, in other words, a large energy barrier to inhibit tumbling motion. There is also the possibility that the protein molecules attract around themselves a mantle of one or more of the phospholipid components of the bilayer, with which they associate relatively strongly. Practically nothing is known about the degree of specificity of protein-lipid interactions in membranes, or yet of their character and mechanism. There is scope for reasonable conjecture, not to say notions on the wilder fringes, involving such extravagant concepts as inside-out globular proteins.

One of the more conservative experimental approaches is to find a membrane enzyme which can be reversibly stripped of its lipids, and then use its activity after recombination with selected lipid components to assess the degree of specificity of its association with the bilayer constituents. In general the upshot has been that the requirements are only moderately specific. A very clear-cut and instructive new example of such a system comes from Garland and Cori (*Biochemistry*, **11**, 4712 ; 1972), working with the microsomal enzyme, glucose-6-phosphatase. The active preparations are lipoprotein particles, which lose their activity on treatment with phospholipases. These enzymes, however, are not nice to use in such a situation, on account of requirements for calcium ions or of the release of hydrolytic products, both of which complicate the interpretation in a disagreeable manner. What Garland and Cori have found is that under carefully defined conditions, non-ionic detergents, and in particular deoxycholate at sufficient concentration, permit reversible separation of the lipid. When the resulting mixture is applied to a 'Sepharose' column, the matrix of which excludes only very large particles, some of the protein, together with all the enzymatic activity, elutes in the void volume, but the bulk of the protein and nearly all the phospholipid are retarded, and emerge with different elution profiles. This protein fraction, almost free of lipid and inactive, can be completely reactivated by addition of new lipid. This only works, however, if some deoxycholate is kept in the mixture, or to

Structural Probes and Peptides

IN next Wednesday's *Nature New Biology* (January 17) Beyer, Craig and Gibbons describe an extension of the application of fluorescent probes of protein structure to oligopeptides. The dyes, anilinonaphthalene sulphonic acid and *p*-toluidinylnapthalene sulphonic acid, are widely used as markers for local conformational changes in proteins. Their fluorescence is greatly enhanced when they bind on low-polarity sites, such as active-centre cavities, in the protein molecule. Using thin-film dialysis and fluorescence spectroscopy Beyer *et al.* studied the interaction of one of the dyes (TNS) with the cyclic antibiotic peptides, tyrocidines A, B, and C, gramicidin S–A, and bacitracin A. In thin-film dialysis the escape rate of a given molecule through a membrane is proportional to the concentration of that molecule on the inside.

With a membrane that passes free TNS much faster than the oligopeptides, Beyer *et al.* found that the escape of TNS is noticeably retarded by all the above peptides, as well as linear peptides, but not by succinyltyrocidine B. This indicates that coulombic interactions play a significant part in the binding process, for the effect of succinylation is to convert a positive charge, on an ornithine side chain, which would interact favourably with the sulphonyl group of the dye, into a negative charge.

In spite of the fact that there is interaction with the whole range of peptides, fluorescent enhancement was observed only in the presence of the tyrocidines. These are distinguished from the others by their tendency to self-association in aqueous solution. The associated form evidently provides an environment in which the TNS may be sequestered, so as to generate large quantum yields.

© 1973 Nature Publishing Group