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.

a lesser extent, if the phospholipids are first sonicated.

If, on the other hand, the bile salt concentration is too high, the activity falls catastrophically. Different phospholipids varied considerably in their ability to engender reactivation. The mono-unsaturated phosphatidylcholine is most effective, and generates full activity at half the concentration of total lipid present in the original preparation. The saturated version, dipalmitoylphosphatidylcholine, by contrast induces no activity whatever. The doubly unsaturated dioleyl derivative behaves similarly to the mono-unsaturated species. There is some precedent for this type of specificity relationship in regard to other membrane enzymes, but the mechanism is obscure. The role of the bile salt in promoting reactivation must be supposed to reside in its ability to destabilize the phospholipid micelles, and so make monomers available for reaction with the protein.

A different path towards the study of lipid-protein interactions has been pursued by Marchesi and his colleagues, who have been studying the red cell membrane glycoprotein (so-called glycophorin), which carries cell surface receptor groups. This molecule probably has a molecular weight of about 50,000, contains 60 per cent by weight of carbohydrate, and, so the story goes, is the most integral of membrane proteins, in that it projects right through the bilayer, whereas the bulk of red cell membrane proteins are to be found at the inner surface.

Segrest *et al.* (*Biochem. Biophys. Res. Commun.*, **49**, 964 ; 1972) have now sequenced a large tract of this molecule, which is evidently the very part that is normally lodged in the lipid interior of the bilayer. They have obtained cyanogen bromide and tryptic peptides with a good overlap. Two fragments are sialoglycopeptides, and therefore from the N-terminal end of the protein, which lies on the outer cell surface. Another is the C-terminal tract, which overlaps with a long tryptic peptide. Between them they define a sequence of fifty-one residues, all but the C-terminal end of which must be presumed normally to reside within the membrane bilayer. Twenty-three successive residues in the sequence are hydrophobic. At either end there is a considerable cluster of charged side chains, and it is reasonable to infer that these may be the parts of the protein that project into the aqueous media on the inside and outside of the cell. Segrest *et al.* refer to evidence of their own, as yet undivulged, that the hydrophobic stretch is an α-helix. Its length in this case would be some 35 Å, which is about right to span the bilayer. If the authors can confirm this model, it will clearly be a result of great interest.

LASSA VIRUS

A New Disease of Man?

from our Medical Virology Correspondent

THE name "arenovirus" defines a group of RNA viruses with a mean diameter of 110–130 nm, a dense well-defined envelope covered with projections and containing a variable number of electron-dense granules, giving a sandy appearance from which the generic name is derived (*Arenosus*, L. sandy). Other characteristics separate the arenoviruses from RNA lipid solvent-sensitive viruses. The group includes lymphocytic choriomeningitis virus, the Tacaribe complex viruses (haemorrhagic fever viruses of South America) and the recently described Lassa virus, all of which cross-react antigenically.

The dramatic story of Lassa fever began when two missionary nurses from Lassa, in north-east Nigeria, died in 1969 from a mysterious illness, and a third nurse, who was gravely ill, was flown to the United States. This nurse recovered and convalescent plasma from her was effective in the treatment of a laboratory worker, who acquired the infection while working with tissue cultures infected with blood from these patients (J. D. Frame *et al.*, *Amer. J. Trop. Med. Hyg.*, **19**, 670 ; 1970). The virus was isolated and characterized (S. M. Buckley and J. Casals, *ibid.*, 680) and Lassa fever was established as a new virus disease of man.

A further outbreak of Lassa fever, with a high mortality of 52 per cent among twenty-three patients admitted to hospital, was reported in Jos, Nigeria, in 1970 (H. A. White, *Trans. Roy. Soc. Trop. Med. Hyg.*, **66**, 390 ; 1972). Dr Jeannette M. Troup, who performed two autopsies while investigating Lassa

Third Type of Pyroclastic Rock

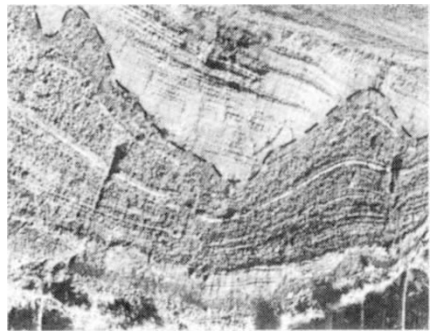
PYROCLASTIC rocks—fragmental volcanic products ejected from volcanoes in explosive events—have usually been divided into two categories, fall deposits and flows. But in next Monday's *Nature Physical Science* (January 15) Sparks and Walker propose a third category, that of ground surge deposits. This category includes some deposits at present classified as pyroclastic flows—frothy, gas-filled glassy lava which bubbles from a volcanic vent during eruptions—and some deposits of *nuées ardentes*, which include the glowing avalanches that dominate the popular picture of volcanic eruptions.

The ground surge deposits are thus characterized by flows along the surface of the Earth, and are clearly distinguished from fall deposits, which show the effects of their passage through the air after ejection, for example in their internal stratification (see figure). But why is it necessary to distinguish between the new category and the pyroclastic flow category?

Active pyroclastic flows are believed to be fluidized by dissolved magmatic gas or by air trapped beneath the advancing flow. They travel as a dense mass, and pumice floats to the top of the flow, where it is preserved when the lava cools to form solid rock. These characteristics result in a structure which mantles the topography around the volcanic vent with a roughly uniform layer of rock.

Although ground surge deposits also mantle the topography, their thickness is not uniform—"they show a pinch and swell structure", as Sparks and Walker put it. Furthermore, they have an internal stratification which is not parallel to the bottom and top of the layer, and they extend out to only a few kilometres from the source. They are relatively thin—less than 1 m compared with the thickness of 10 m or more commonly associated with pyroclastic flows—and they can occur on relatively steep slopes ($>10°$) where pyroclastic flows do not solidify.

The overall picture painted by Sparks and Walker is one in which explosive volcanic activity produces, in addition to the ejecta thrown high into the air, both a dense flow, which chiefly follows the valleys, and a relatively low density ground surge, or "ash hurricane", akin to the ground surge generated in a nuclear explosion. The resulting thin ground surge deposit is very susceptible to erosion, and survives only when covered by other volcanic deposits ; that explains why it has taken so long for the ground surge to be recognized as a separate category. Finally, the pinch and swell structure can be accounted for by a wave pattern, with wavelengths found so far in the region 4 m to 45 m.



Pyroclastic fall deposits. Dashed line is an erosional unconformity ; the fall deposit above it shows internal stratification common among such deposits.