FLUORESCENT PROBES

Time Resolved Redshift

from a Correspondent

STUDIES on the conformation and polarity of biological enzymes and membranes have made increasing use of fluorescent probes in recent years. Although a large number of articles have appeared in which probe interactions with biological substrates were examined, comparatively few investigations have been concerned with the behaviour of typical fluorescent molecules in well-defined environments. A welcome addition to this last category is an important contribution from Ware's group (S. K. Chakrabarti and W. R. Ware, *J. Chem. Phys.*, **55**, 5494; 1971), who report temperature and time-dependent fluorescence measurements on the well known fluorescence label, 1-anilino-8-naphthylene sulphonate (ANS).

Chakrabarti and Ware are primarily concerned with the redshift of the ANS fluorescence band maximum in polar solvents. For a fluorescent molecule which is free to diffuse in solution, these spectral shifts are thought to arise from solvent reorientation about the excited state that is required to accommodate the transient changes in electronic structure which occur upon light excitation. (The excited singlet state of ANS is more polar than the ground state as a result of the charge-transfer nature of the spectral transition.)

Ware has developed an instrumental technique which can be used to measure the transient changes with time over the complete fluorescence spectrum. This makes it possible to study kinetically the dynamic processes which determine the spectral change, namely the solvent environment and its relaxation subsequent to excitation of the absorbing molecule.

Chakrabarti and Ware find that time dependent spectral shifts for ANS are observed only in the temperature range from -70° to -170° C in *n*-propyl alcohol. At higher temperatures they see the fully relaxed time independent spectrum normally found at room temperature, with wavelength maximum at 480 nm.

In general, the time required to approach the relaxed spectrum gets longer as the temperature is decreased or the viscosity of the solvent is increased. At temperatures less than -170° C, no time dependent spectral shifts were observed, and the fluorescence intensity maximum is blue-shifted to 438 nm. This indicates that under these conditions the time for reorientation of solvent molecules is too slow to affect the emission properties of the fluorescent species. In essence, each emitting ANS molecule is in an

environment with nearly the Franck-Condon solvent configuration.

These results are quite similar to those reported earlier for the 4-aminophthalimide-propyl alcohol system (W. R. Ware *et al.*, *ibid.*, **54**, 4729; 1971), except for the spectral behaviour at very short times after excitation. The fluorescence spectra of the aminophthalimide showed evidence of a sub-nanosecond relaxation process which occurred even at low temperatures. The rapid effects were discussed in terms of interactions within an electronically excited solute-solvent complex.

These time dependent measurements emphasize the ambiguity which exists when spectral shifts of ANS or similar molecules are used as probes for the polarity of their surroundings. Only when "molecular motion akin to solvent reorientation" occurs will the probe function reliably as a polarity indicator. Specifically, interpretation of experimental results is expected to be more difficult when ANS is bound to a biological substrate. Some binding sites may exclude solvent molecules (see S.

Ainsworth and M. T. Flanagan, *Biochim. Biophys. Acta*, **194**, 213; 1969), and others may place geometrical constraints on the probe, thereby changing its emission properties. It will be interesting to see if time resolved emission spectroscopy can be used to simplify the interpretation of spectral shifts demonstrated by fluorescent probes.

RADIOLOGICAL PROTECTION

In the Event . . .

from a Correspondent

THE problems of radiation exposure associated with nuclear weapons were discussed at a meeting of the Society for Radiological Protection at Imperial College, London, on January 11. The characteristics and effects of nuclear weapons were described by Mr J. C. Cotterill (Home Office) who considered that the immediate damage by radiation exposure from medium and high explosive nuclear bombs would be within the range of destruction created by the blast and thermal effects. But radio-

B and T Cells Separated

VARIOUS means have been adopted to produce populations of cells in which the proportion of B and T cells has deliberately been adjusted. In earlier, cruder, experiments lymphoid cell populations from either neonatally thymectomized or adult thymectomized and irradiated mice have been taken to be selectively depleted of T cells and therefore rich in B cells. But it is now realized that there are many residual T cells in these animals and that their use is thereby restricted.

The discovery and description of athymic (NuNu) mice provided a source of very nearly pure B cells, but this particular strain of animal is both difficult to rear and is at present not inbred. Miller and Sprent (*Cell. Immunol.*, in the press) have devised a more esoteric system whereby CBA thoracic duct lymphocytes are injected into heavily irradiated CBA $\times$ C57Bl mice. The cell population which, subsequently, can be recovered from the thoracic ducts of the recipients is a nearly pure T cell population but is also heavily committed to an anti-C57Bl response. Perhaps the best method so far has involved the use of anti- θ antisera which are capable of killing T cells in the presence of complement. The use of such selective sera has, however, been criticized on the grounds that they may sometimes contain anti-B cell antibodies, autoantibodies, and fail to eliminate transformed T cells. Basten *et al.*, in *Nature New Biology* next Wednesday (February 9), now provide an elegant method for the separation of B

and T cells which should be generally applicable to normal mice in any reasonably well-equipped laboratory.

It was first established that, when mouse thoracic duct lymphocytes were incubated sequentially with either mouse anti-fowl gamma globulin or mouse anti-human gamma globulin and then with either iodinated FyG or HGG, 15 to 20 per cent of the cells bound the radioactive material. An autoradiographic method was used to show this. As expected, cells incubated with anti-FyG only bound FyG and cells incubated with anti-HGG only bound HGG. Evidence is presented that the binding depends on the formation of antigen-antibody complexes on the surfaces of the binding cells. Basten *et al.* go on to show that, if they pass immunoglobulin coated cells through columns of beads themselves coated with the appropriate antigen, selective retention of those cells which then form surface antigen-antibody complexes can be achieved.

Basten *et al.* demonstrate clearly that they can remove B cells from a lymphoid cell population. They conclude that B cells have weak receptors for immunoglobulin molecules. Formation of an antigen-antibody complex can strengthen the tie between an attached immunoglobulin and its carrier B cell in such a way that the bead-antigen-antibody-cell complex is firm enough to withstand the forces of gentle elution. Subsequent discharge of B cells from the column was achieved by vigorous shaking with an appropriate medium.