human beings, have been shaped by natural selection to behave and to feel in certain ways.

BIOLUMINESCENCE

Identified Glow

from our Molecular Biology Correspondent

"I CAN think of nothing eerier/Than flying around with an unidentified glow on a person's posterior." Thus Ogden Nash, on the subject of the firefly. Little, one supposes, did he guess that the glow arises from the oxidation of the benzothiazole derivative, luciferin, by molecular oxygen after it has been bound to the enzyme luciferase and then adenylylated by ATP and magnesium. This much is now established, and it is also known that the dehydrogenated derivative, which contains one more double bond, dehydroluciferin, will compete with luciferin, and undergo the same chemistry, but for the last step, the enzyme-bound adenylate not being reactive to oxygen. It undergoes in the course of binding some interesting and revealing changes in fluorescence, and to see more deeply into the nature of the interaction of luciferin with the enzyme, Bowie, Horak and De Luca (*Biochemistry*, **12**, 1845; 1973) have now synthesized a further analogue, which does not react with ATP. This is the alcohol, in which the carboxyl group of dehydroluciferin is replaced by a hydroxymethyl group, and is referred to as dehydroluciferol. Its spectroscopic properties are similar to those of its analogues, and it binds with the identical stoichiometry and a similar association constant to luciferase. In the bound state it is unaffected by ATP. Reciprocal plots show strong inhibition of the luciferase reaction.

In the accompanying article, Bowie *et al.* (*ibid.*, 1852) examine the nature of its interaction with the enzyme. In the first place, it must be noted that the fluorescence spectrum of luciferin and its analogues in aqueous solution shows a maximum in the region of 550 nm, which is characteristic of the phenolate form, in which the aromatic hydroxyl group is ionized. The excitation spectrum, however, is that of the neutral unionized molecule. This situation is not unique, for acids tend to become stronger on electronic excitation, and the proton can then dissociate during the lifetime of the excited state, leading to emission from the ionized species. When, however, the molecule is bound to the luciferase, a second smaller maximum, at about 425 nm, appears in the fluorescence spectrum, which arises from the unionized form. Thus the loss of the proton in the excited state is inhibited by the environment of the active site. There is at the same time a blue-shift in the phenolate emission, and both these phenomena are easily enough interpreted in terms of the location of the chromophore in a hydrophobic cavity. When now ATP with magnesium is added, a large increase in the relative fluorescent intensity at 425 nm ensues, indicative of a change in the character of the binding site, or of an increase in the fraction of ligand bound. In fact it turns out that both factors contribute, for titration in the presence of ATP and the corresponding binding plot show that the binding constant in these conditions is up by a factor of ten, but the resulting increase in the proportion of bound ligand is insufficient to account for the intensity change. The conclusion is that the quantum yield of the fluorescence of the bound form is increased by a factor of two or more.

Bowie *et al.* have also tried the effect of AMP, which is known to stimulate the chemiluminescence. It leads to a blue-shift in the phenolate emission, and the phenol peak is totally expunged. The polarity of the site can be matched by a solvent of low dielectric constant, chloroform, in which the phenolate emission of dehydroluciferol, which can be provoked in this medium by addition of a base to act as a proton acceptor, appears at the same wavelength. The AMP evidently also causes a change in the location of interacting groups in the binding site, in such a sense as to facilitate the proton transfer process. To complete the picture, the fluorescence decay kinetics have been observed. The initial formation of the unionized excited state is followed in the first few nanoseconds by the appearance of the excited phonolate. The proton transfer rate is diminished in the luciferase complex, and still more in the presence of ATP. These rates are reflected in the phenol excited state lifetime, which is increased on ATP binding, but marginally decreased by AMP. The authors suggest that the very high quantum yield of the bioluminescence may well involve the protection of the emitting species from quenching processes, and from hydrolytic destruction, by the hydrophobic milieu.

Bioluminescence of course occurs not only in fireflies but also in some bacteria, fungi and various creatures of the deep. In some of the luminescent coelenterates the mechanism is more or less similar to the firefly, with oxidation of luciferin on luciferase by molecular oxygen. This occurs, for example, in the class Anthozoa. The complicating feature of this system, however, has been that the chemiluminescent emission *in vitro* occurs at 490 nm whereas the intact animal lights up green, with a maximum at 509 nm, and some vibrational structure around 540 nm. An explanation has been contrived in terms of energy transfer to another chromophore, and indeed the search for such an acceptor led to the isolation of a green-fluorescing protein. Anderson and Cormier (*J. Biol. Chem.*, **248**, 2937; 1973) reasoned that if the excitation transfer is to proceed with high efficiency, the luciferase and the fluorescent protein should be packaged together at high concentration. The supernatant of homogenates was then discovered to produce the 490 nm luminescence, whereas the pelleted fraction emitted at 509 nm. This particulate matter could be purified by sucrose gradient centrifugation, and was found to contain luciferase, green-fluorescent

Membrane Properties of Tumour Cells

IN *Nature New Biology* next Wednesday (June 13) Nicolson enlarges on the differences in agglutinating ability between normal and transformed cells. It has been known for some years that a number of plant lectins, which specifically bind certain saccharides, will agglutinate transformed but not normal cells.

This was attributed at the outset to the exposure of "cryptic" binding sites in the process of transformation. Since then evidence has accumulated that agglutinability of the cells is determined by the ease with which the glycoprotein receptors are able to diffuse in the plane of the membrane, and create clusters that promote strong interaction with another cell.

This cluster formation can be observed by labelling with ferritin-bound or fluorescent anti-lectin antibodies. It has also been reported that tumour cells will agglutinate with concanavalin A, a typical lectin, at room temperature, but not at low temperatures. It would be expected that this effect results from a dependence of the fluidity of the membrane on the temperature, which is what Nicolson has now demonstrated.

Concanavalin A-treated cells labelled with fluorescent anti-concanavalin antibodies at 0° C show uniform surface fluorescence. Normal cells first incubated at 22 or 37° C and then cooled to 0° C look similar. On the other hand transformed cells treated in this way show fluorescent patches. Labelling at 0° C after fixation with formaldehyde inhibits the temperature-induced formation of clusters. Thus the distinctive behaviour of tumour cells towards lectins is evidently a function of a greater fluidity in the membrane bilayer, and this effect is not expressed at low temperature.