cients are measured at low intensity while optical transparency will be induced at high intensity; the latter effect, however, is comparable for the two gases used here.)

We fine *no* reaction in case (a) above, while (b) proceeds at nearly the same rate and with the same products as if SF_6 were absent. In fact, a null result is obtained at the 949-cm^{-1} frequency with SF_6 pressures through 20 Torr, or with laser power increased to 8 W. By comparison, at 935 cm^{-1}, we find the reaction rate grows as the eleventh power of laser intensity.

In other experiments, various amounts of helium were added to 100 Torr of CF_2Cl_2. The reaction rate was virtually uneffected for He pressures up to 40 Torr. Since the latter addition increases the macroscopic thermal conductivity by a factor of 5, one expects a significant decrease in the temperatures produced. Because a thermal reaction rate would vary exponentially with reciprocal temperature, the experimental result may be considered as further evidence against a single heating mechanism.

The vibrational mode of CF_2Cl_2 excited in these experiments is believed to represent a rocking motion of the CF_2 group against the Cl_2 group[4,5] (or vice-versa). We note also that under isothermal conditions, CF_2Cl_2 is reported to be entirely stable below 700°, decomposing entirely at 900° to products other than those found here,[6] while Freon 114 decomposes entirely[7] at 500°.

Freon 12 has been studied[8] as a saturable absorber for laser mode locking. At the pressures used (~1 Torr) the reaction rate is too small for convenient measurement, but it may be large enough in the long run to prevent the application mentioned.

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On the Fluorescence of Bilirubin[1]

Sir:

Recently we reported on the low temperature fluorescence of biliverdin and biliverdin dimethyl ester,[2] both of which exhibited a two-fluorescence band system with emission maxima at 725 and 480 nm. At that time the fluorescence of *free* bilirubin (**1**) was difficult or impossible for us to detect. Indeed, whether free bilirubin fluoresces or whether the fluorescence can even be detected has been the subject of controversy;[3] whereas, it has been clearly established that bilirubin fluoresces when it is *bound* to human serum albumin,[3,4] rabbit, horse, porcine, or sheep albumin.[3] In order to study the fluorescence of free bilirubin, we resorted to the use of micelle forming detergents in aqueous solution[5] and in EPA solvent. We report herein the first observation of free bilirubin fluorescence in a micellar environment at room temperature and at 77°K.

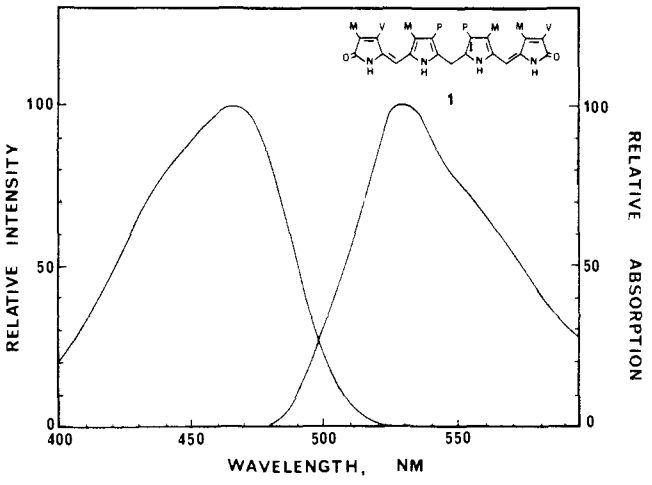

Figure 1. Room temperature fluorescence and excitation spectra of bilirubin (**1**) (5.2 mg) in 100 ml of water containing 20.5 mg of cetyltrimethylammonium bromide. Excitation wavelength and bandpass were 440 and 10 nm, respectively: M = CH_3, V = $CH{=}CH_2$, and P = CH_2CH_2COOH.

Bilirubin (**1**)[6] is insoluble in water and common organic solvents, although it exhibits very limited solubility in a few organic solvents, e.g., chloroform, benzene, and dimethyl sulfoxide. However, it is readily solubilized in water and some organic solvents in the presence of a detergent such as cetyltrimethyl ammonium bromide (CTAB).[7a] Spectroquality EPA[7b] (ether-isopentane-ethanol, 5:5:2) and glass distilled water were used as solvents in this work. In distilled water containing 5.2 mg % **1** and 20.5 mg % CTAB we observed room temperature fluorescence emission[8] at 530 nm from **1** when the excitation wavelength used was 440 nm[9] (Figure 1). When the excitation wavelength used was the more typical 390 nm, only an extremely low level of and barely detectable fluorescence emission was observed. These findings support the disputed[3] observation of Beaven et al.[4] of an extremely weak fluorescence from **1** in aqueous solution at pH 8.4. The fluorescence of **1** at room temperature, in neutral solution with added detergent and without albumin, is clearly established.

The fluorescence of bilirubin in nonaqueous solvents has not been published heretofore, although observations of bilirubin fluorescence (λ 525 nm) have been made for a methanol (with added ammonia) glass solution.[10] We have found fluorescence emission[11] from **1** in EPA glass at 77°K with and without added detergent (CATB)[9]: in 10^{-5} to 10^{-6} *M* solutions of **1** alone and in the presence of monomeric (10^{-4} to 10^{-5} *M*) CATB as well as excess (10^{-3} *M*) CATB. By varying the concentration of CATB, in EPA the fluorescence λ_{max} shifts from 530 to 505 nm from pure EPA to 46.4 mg % CATB in EPA (Figure 2), respectively. Shore and Turro[12] have used this type of spectral shift to deduce the critical micelle concentration (cmc) of a host detergent from the inflection point in the fluorescence vs. detergent concentration plot. They determined a cmc $\simeq 8.8 \times 10^{-4}$ *M* for cetyltrimethylammonium bromide using 11-[3-hexyl-1-indolyl]-undecyltrimethylammonium bromide as a fluorescent probe. Similarly, by using **1** as a fluorescent probe, we have determined the CATB cmc to be $\sim 2 \times 10^{-4}$ *M*.

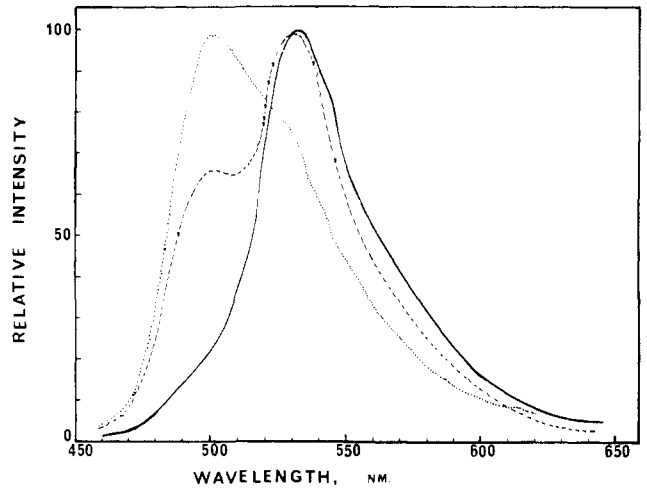

Figure 2. Variation in the fluorescence λ_{max} of bilirubin at 77°K in EPA (ether-isopentane-ethyl alcohol 5:5:2) solution containing varying concentrations of cetyltrimethylammonium bromide: 2.0 mg bilirubin in 25 ml of EPA with no added CATB (——); with 0.6 mg of added CATB (- - -); and 0.05 mg of bilirubin in 25 ml of EPA with 11.6 mg of added CATB (···). Excitation wavelength and bandpass were 390 and 10 nm, respectively.

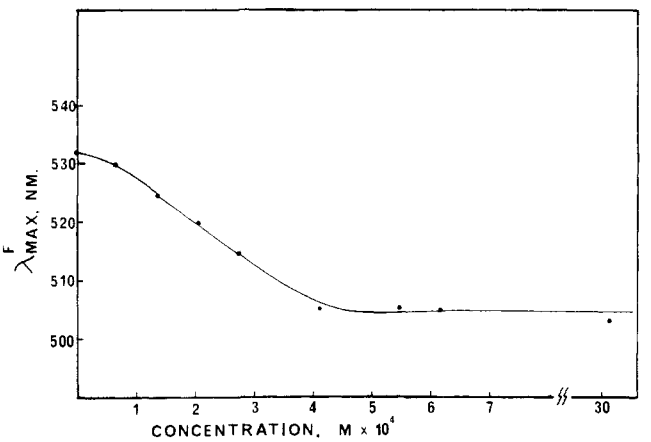

Figure 3. Plot of the fluorescence λ_{max} of bilirubin vs. various concentrations of cetyltrimethylammonium bromide in EPA solutions at 77°K.

The structure of the micelle is as yet uncertain and whether bilirubin is suspended as a monomer is unknown.

It may be noted in Figures 1 and 2 that **1** in aqueous detergent fluoresces at λ_{max} 525 nm; whereas, in EPA-detergent, the λ_{max} is 505. We attribute these differences to differences in conformation (and ionization?) of **1** induced by their different micellar environments. Thus, we propose that, in water, the ionic part of CATB is on the outside and the lipophilic hydrocarbon part of CATB is on the inside of the micelle and bilirubin resides in a strongly lipophilic environment. On the other hand, in EPA, we propose that the relative polarity of the micelle is reversed with the lipophilic surface on the outside and the polar ionic surface on the inside. In the latter, **1** is exposed to a strongly ionic, hydrophilic environment; whereas, in the former it is exposed to a nonionic, lipophilic environment; hence, differences in conformation of **1** are to be expected.

In light of the foregoing, one might therefore expect bilirubin in a lipophilic environment such as EPA alone to exhibit similar fluorescence behavior as in aqueous detergent. Indeed, such is the case as may be noted from a comparison of Figures 1 and 2. Further evidence supporting the notion of similar conformation(s) of **1** in similar environments comes from the observation that **1** also fluoresces at λ_{max} 525 nm in (lipophilic) chloroform solvent at room temperature.

Whether the structure of **1** in our lipophilic environments is akin to that internally hydrogen bonded structure described by Kuenzle et al.[13] cannot be ascertained from our data. We speculate that it might be an important contributor. Furthermore, although little is known of the structure of **1** bound to albumin,[14] it too fluoresces with λ_{max} near 525 nm[3,4] which is suggestive of similar conformation(s) for **1** here as in water-CATB, EPA, and chloroform without albumin.

In contrast to the fluorescence behavior of **1** in nonpolar solvents, when a solvent with a high dielectric constant, acetonitrile, is employed, the fluorescence λ_{max} shifts to ~505 nm with a broad shoulder on the long wavelength side. This behavior is in keeping with that found for **1** in EPA with high concentrations of CATB. However, the fluorescence $\lambda_{max} \simeq 530$ nm (with shoulder at $\lambda_{max} \simeq 505$ nm) for **1** in EPA + sodium methoxide is not entirely expected from the preceding arguments and doubtless reflects an ionization phenomenon (amide as well as carboxyl?) in addition to possible conformational changes.

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Depolarized Light Scattering and Carbon Nuclear Resonance Measurements of the Isotropic Rotational Correlation Time of Muscle Calcium Binding Protein

Sir:

Molecular reorientation is described by the Debye model as random steps of small angular displacements. For macromolecules, the rotational diffusion coefficient, D, can be calculated hydrodynamically from the Stokes-Einstein equation.[1] Rotational Debye motion causes fluctuations in the laboratory reference frame polarizability, if a molecule is optically anisotropic. Depolarized light scattering mea-