Luminescence of Bilirubin

By RAYMOND BONNETT*
(*Department of Chemistry, Queen Mary College, Mile End Road, London* E1 4NS),
and JOHN DALTON and DAVID E. HAMILTON
(*Department of Chemistry, City of London Polytechnic, 31, Jewry Street, London* EC3N 2EY)

Summary The luminescent state of bilirubin in EPAF glass at 77 K is very short lived (<5 ns) and is therefore regarded as the singlet; there is evidence that this emission occurs from a species of bilirubin distinct from that predominating in solution at room temperature.

THE photochemistry of bilirubin is of particular interest because of its relevance to the phototherapy of neonatal hyperbilirubinemia.[1] Little is known, however, about the excited states of bilirubin. Bilirubin is reported to be non-fluorescent,[2] and certainly does not luminesce visibly at room temperature when irradiated at 365 nm. We recently reported, however, that in various polar solvents as glasses at 77 K a greenish emission could be observed at *ca.* 525 nm, and the question of the multiplicity of the luminescent state was raised.[3]

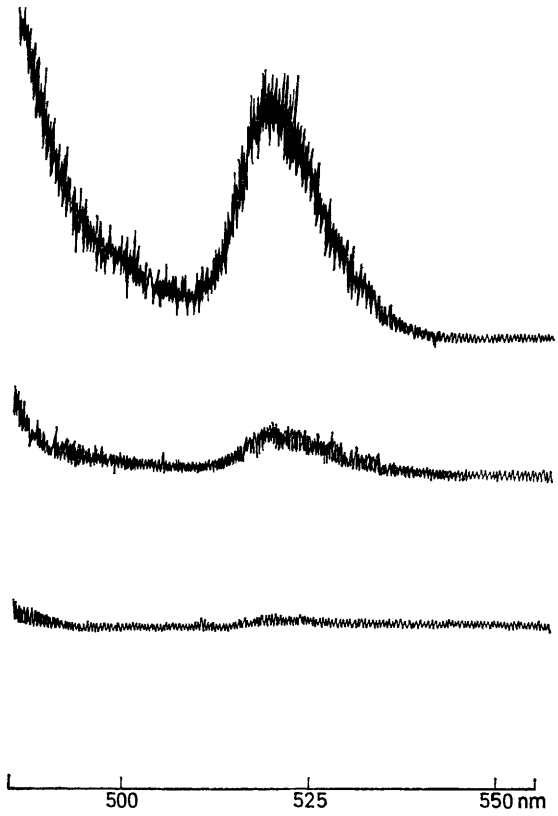

FIGURE. Fluorescence emission spectrum of bilirubin in degassed EPAF glass at 77 K recorded at constant gain 1 (top), 10, and 20 (bottom) ns after excitation by a rapidly pulsed lamp filtered to remove radiation of wavelength > *ca.* 500 nm.

The luminescence spectrum of bilirubin in degassed EPAF [ether–isopentane–ethanol–dimethylformamide (6-:5:3:0·5)] glass at 77 K was recorded (Figure) at periods of 1, 10, and 20 ns after excitation (< 450 nm) with a rapidly pulsed lamp. The decay rate of the 520 ± 5 nm emission maximum was insignificantly different from that of the excitation pulse, implying an emission half-life of < 5 ns under the above conditions. This characterises the luminescence as fluorescence rather than phosphorescence. The apparent quantum yield,[4] by comparison with fluorescein at room temperature, was *ca.* 0·05.

The excitation spectrum, monitored at the 520 nm emission maximum, showed a single rather sharp maximum at 472 ± 5 nm, and differed markedly from the absorption spectrum (in EPAF at 293 K) which possessed a broad band at 450 ± 5 nm. However, the absorption altered reversibly on cooling to 77 K, with the development of a maximum at *ca.* 470 nm. The comparison of emission and absorption spectra was repeated at 215 K using dichloromethane and dimethylformamide as solvents. In the former the 450 nm absorption band showed no detectable change from its shape at 293 K, and fluorescence could not be detected. In the latter a change in absorption band shape similar to that observed in EPAF was accompanied by a fluorescent emission at 520 ± 5 nm.

One interpretation of these observations is that in some solvents, notably polar ones, a second species of bilirubin is present in equilibrium, and is favoured at lower temperatures; it is then supposed that it is this species that is fluorescent. This second species could arise by aggregation and/or by specific solvation resulting in a particular conformational preference becoming evident at the lower temperature.

We thank S.R.C. for financial support, and the I.L.E.A. for a research assistantship (to D.E.H.).

(*Received, 8th May* 1975; *Com.* 528.)

[1] R. J. Cremer, P. W. Perryman, and D. H. Richards, *Lancet*, 1958, (i), 1094.
[2] E. Miedziejko and D. Frackowiak, *Photochem. Photobiol.*, 1969, **10**, 97.
[3] R. Bonnett and J. C. M. Stewart, *J.C.S. Perkin I*, 1975, 224.
[4] A recent report gives a quantum yield of 0·11 for the potassium salt of bilirubin in ethanol (77 K): I. B. C. Matheson, G. J. Faini, and J. Lee, *Photochem. Photobiol.*, 1975, **21**, 135.