lowest maximal growth temperature (strain 58) caused detectable biochemical changes of the substrate within the given exposure time. But even in this case, the rate is strongly reduced as compared to that in the laboratory controls.

These data suggest that, superimposed on a quantitative reduction of the rate of biochemical activity, the increased hydrostatic pressure may exert an effect on the cells, raising the minimal growth temperature. When this increase exceeds the environmental temperature, the cells will become inactive. This effect would be similar to, but not necessarily biochemically linked to, the observed increase in temperature tolerance of bacteria (*4*) and of isolated enzymes (*8*) when exposed to similar increases of pressure.

We now propose the hypothesis that, in an environment of low temperature, an increasing pressure will eliminate growth and biochemical activity of bacterial types successively as their minimal growth temperatures are shifted toward, and ultimately surpass, the environmental temperature. Thus, psychrophilism of our isolates at normal pressure may be defined as an expression of adaptability to the combined effect of high pressure and low temperature. Or, in other words, psychrophilic bacteria would not necessarily react as psychrophiles in the deep sea. Laboratory experiments in this direction are under way.

Our hypothesis may be further supported by the fact that in marine sediments from depths of 1300 and 2600 m extremely obligate psychrophilic bacteria that exhibited maximal growth temperatures between 8° and 15°C have been isolated. These types are not found in shallower waters where obligate psychrophiles with maximal growth temperatures between 17° to 24°C are present (*9*). Strain 58 belongs to the latter group but appears to have the potential of being biochemically active at 2° to 3°C at a depth of 4300 m.

In seawater collected at a depth of 200 m (17.6°C), mesophilic bacteria were predominant while obligate psychrophilic bacteria were absent (*9*). This may explain the low absolute rates of degradation in these samples when exposed to deep-sea conditions.

One obvious implication of our findings concerns the use of the deep sea as a dumping site for organic wastes. The relatively low rates of microbial activity at deep-water conditions appear to render this way of waste disposal very inefficient compared to the degradation of organic wastes in land-disposal sites or in treatment plants. Accumulations of waste materials or intermediate decomposition products in the deep sea appear rather uncontrollable. Bruun and Wolff (*10*) mention the common recovery of water-logged wood materials from deep-sea dredgings even far from land.

Normally, few solid organic materials, produced on land or in the sea, can be expected to reach the deep sea without passing surface waters or shallow-water sediments where considerable degradation occurs. If this step during offshore disposal were eliminated, it seems possible to trap substantial amounts of nutrients in solid form in the deep sea, and thereby remove them from natural or technically enhanced recycling processes. The notion of fertilizing the sea with man-made wastes might not be applicable with regard to deep-sea dumping.

Although neither microbial population collected from surface or deep waters showed appreciable activities when exposed to deep-sea conditions, our data do not entirely disprove the possibility of long-term enrichments in deep-sea sediments. Whether or not adaptive processes occur, the rates of oxygen supply and microbial degradation activities will determine the extent to which anaerobic conditions will arise, with possible elimination of the benthic nonmicrobial fauna.

HOLGER W. JANNASCH
Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543
KJELL EIMHJELLEN
Department of Biochemistry, The Technical University of Norway, Trondheim
CARL O. WIRSEN
Woods Hole Oceanographic Institution
A. FARMANFARMAIAN
Department of Physiology and Biochemistry, Rutgers University, New Brunswick, New Jersey 08903

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Fluorescence of the Purine and Pyrimidine Bases of the Nucleic Acids in Neutral Aqueous Solution at 300°K

Abstract. *Fluorescence of adenine, guanine, cytosine, and uracil at room temperature in neutral aqueous solution has been detected by means of a digital signal accumulation technique. Corrected emission and excitation spectra are presented and compared with low-temperature data. The quantum yields are, respectively,* 2.6×10^{-4}, 3.0×10^{-4}, 0.8×10^{-4}, *and* 0.5×10^{-4} *when the bases are excited at their low-energy absorption maxima.*

An understanding of the nature of the excited states of DNA is fundamental to an understanding of both electronic energy transfer processes and DNA photochemistry, and luminescence measurements are the most direct way of determining the properties of these states. Earlier work on neutral solutions has been carried out in low-temperature environments where quantum yields are more easily measurable ($\Phi \sim 10^{-1}$). A great amount of data has been accumulated, principally at 77°K in ethylene glycol–water (EG:H_2O) glasses, on the fluorescence, phosphorescence, and electron spin resonance properties of the purine and pyrimidine bases, the corresponding nucleotides, and various DNA's. This work has been reviewed recently (*1*). Extensive work, also at 77°K, has been carried out on homo- and heterodinucleotides and polynucleotides with the aim of understanding

Table 1. Fluorescent properties of the bases at room temperature.

Compound	Concentration ($M \times 10^{-5}$)	pH	Excitation energy (μm^{-1})	Fluorescence quantum yield ($\times 10^4$)	O-O′ energy* (μm^{-1})	Singlet lifetimes† ($\times 10^{12}$) (a)	(b)
Adenine	5	7.3	3.83	2.6	3.56	1.0	8.9
Guanine	8	6.3	3.63	3.0	3.34	1.4	3.0
Thymine (*5*)	5	6.7	3.77	1.02	3.45	0.9	1.5
Cytosine	10	6.5	3.75	0.82	3.49	0.2	0.9
Uracil	8	6.8	3.87	0.45	3.57	0.7	1.4

* Determined by the absorption-emission intersection. † Method of calculation explained in the text.

the role of energy transfer and localization in DNA (*2*).

Because of the effects of temperature on the rates of radiationless transitions and on relaxation from the Franck-Condon state, clear uncertainties exist in the direct applicability of properties of the excited state determined at 77°K to room-temperature behavior. In the biologically significant conditions of neutral aqueous solution at room temperature, however, the lack of luminescence from the constituents of DNA has been consistently reported (*3, 4*). The need and importance of data on the excited states in a room-temperature neutral, aqueous environment is therefore apparent.

Utilizing the techniques of digital signal accumulation and right-angle fluorescence detection from finite absorbing solutions, we have been able to determine corrected relative fluorescence emission and excitation spectra, quantum yields, and O-O′ energies for adenine, guanine, cytosine, and uracil under these conditions [results for thymine are presented elsewhere (*5*)]. Fluorescence and absorption measurements for adenine, cytosine, uracil (all Calbiochem A grade, chromatographically homogeneous), and guanine (Calbiochem CfP grade, chromatographically pure) in triply distilled water were made on an energy-corrected spectrofluorimeter (Turner model 210). Transformations of the data to the form presented in Fig. 1 are described elsewhere (*6*), as is the signal accumulation technique (*5*). To avoid excessive scattering, possible fluorescing impurities, and photochemical reaction, buffers were not used and the reported *p*H of each solution was determined immediately before and immediately after fluorescence measurements.

In all cases the bases exhibit measurable fluorescence after a small number of scans, and corrected relative emission spectra are presented in Fig. 1. Guanine gives the most intense fluorescence (Table 1) and an unstructured emission spectrum with its maximum at 3.05 μm^{-1}, in reasonable agreement with the low-temperature emission (*7*). In contrast with its corrected low-temperature data (*8*), adenine emission is unstructured with its maximum exhibiting a slight red shift to 3.12 μm^{-1}. Such a shift at higher temperatures is expected as a result of the increased efficiency of solvent reorientation in the excited state. Cytosine emission is in good agreement with the uncorrected low-temperature data (*9*) (maximum at 3.19 μm^{-1}), and uracil emission is slightly higher (maximum at 3.24 μm^{-1}) than its low-temperature uncorrected maximum (*9*).

Agreement between absorption and

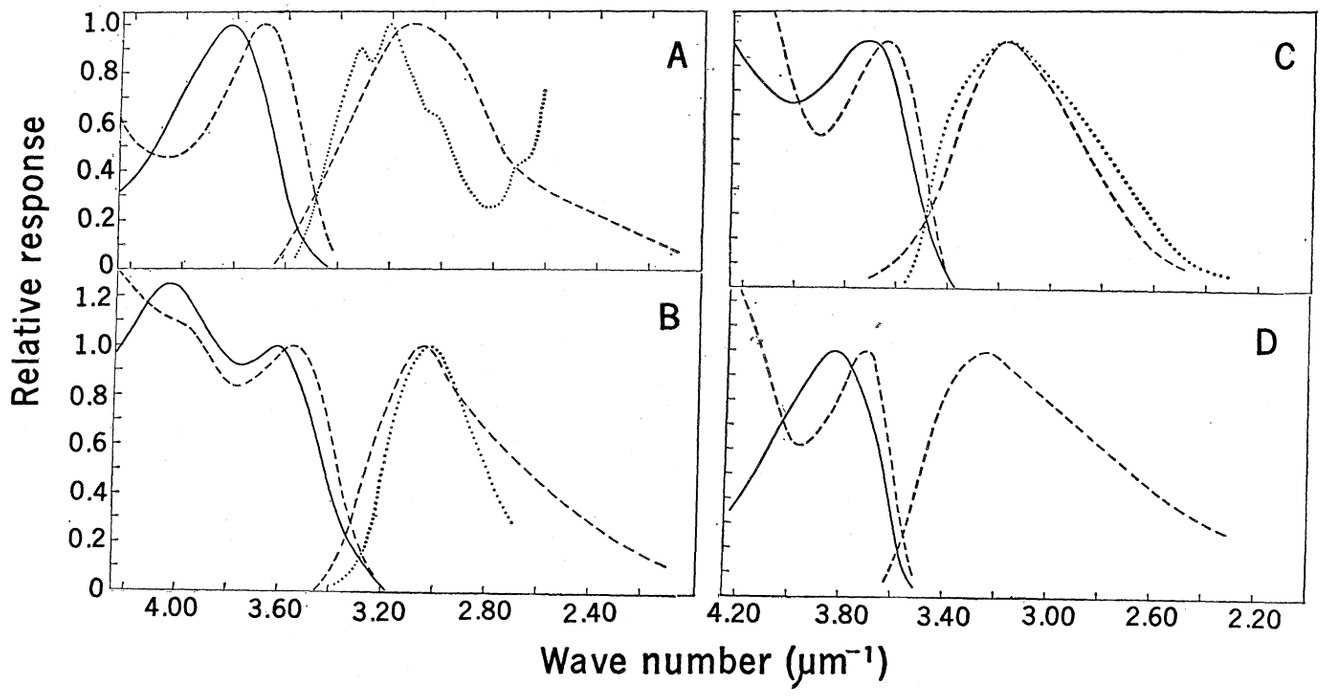

Fig. 1. Corrected fluorescence excitation (dashed line, left) and emission spectra (dashed line, right), absorption spectra (solid line), and emission spectra compared to that in EG:H_2O (1:1, by volume) (dotted line) for four bases. (A) Adenine: two scans (signal-to-noise ratio, > 25); emission compared to that in EG : H_2O at 77°K (*8*), with fluorescence excited at 3.83 μm^{-1} and emission monitored at 3.13 μm^{-1}. (B) Guanine: two scans (signal-to-noise ratio, > 25); emission compared to that at 195°K (*7*), with fluorescence excited at 3.63 μm^{-1} and emission monitored at 3.30 μm^{-1}. (C) Cytosine, four scans (signal-to-noise ratio, > 15); emission compared to that at 77°K (*9*), with fluorescence excited at 3.75 μm^{-1} and emission monitored at 3.13 μm^{-1}. (D) Uracil, four scans (signal-to-noise ratio, > 15); with fluorescence excited at 3.87 μm^{-1} and emission monitored at 3.23 μm^{-1}.

excitation spectra, taken on the same instrument with identical bandwidths, is not evident for any base and may be taken as an implication that there is a variation in the emission yield as a function of excitation energy (*5*). Other examples of such behavior have been reported for the purines and pyrimidines under different conditions. At 170°K in EG:H_2O (70:30, by volume) the relative quantum yields of adenine fluorescence excited at 4.00, 3.73, and 3.57 μm^{-1} are 0.6, 1.0, and 1.9 (*10*), as compared with our values at room temperature and in aqueous solution of 0.54, 1.0, and 2.7. Excitation spectra of guanine (*11*) at acid and alkaline *p*H's, of adenine (*12*) in acid, and of thymine (*13*) in alkali also exhibit a shift relative to absorption similar to our results. Processes that may explain this type of shift include the existence of two tautomeric structures in the first absorption band, only one of which is fluorescent (*5, 10, 12*), the changing efficiency of radiationless deactivation processes as a function of excitation energy (*5*), and emission from an n-π^* or π-π^* state hidden in the red edge of the absorption band (*5, 14*).

The emission quantum yields (Table 1), calculated relative to a PPO (2,5-diphenyloxazole) yield in nitrogen-flushed cyclohexane assumed to be 1.00 (*15*), are sufficiently low to have escaped previous detection. At room temperature the purines fluoresce at least three times more intensely than pyrimidines, whereas at 77°K cytosine fluorescence is reported (*9*) to be as efficient as that of the purines, and thymine fluorescence (*8*) has more than twice the quantum yield of the purines. These changes in the relative order of emission yields as a function of temperature (for example, from 77° to 300°K the guanine yield is reduced 200-fold, but the thymine yield is reduced over 2000-fold) further emphasize the uncertainties in the direct application of low-temperature results to room temperature conditions.

The low observed quantum yields imply correspondingly short singlet lifetimes, $\sim 10^{-12}$ second. This short singlet lifetime has been interpreted as making singlet energy transfer in DNA improbable (*4*) [the estimated transfer rate is 10^{12} sec^{-1} under the most favorable conditions (*16*)]. The possibility, however, of emission arising from a tautomer or excited state hidden in the first absorption band makes the true quantum yields and the oscillator strengths of the fluorescing species somewhat uncertain. Calculated singlet lifetimes are all $\sim 10^{-12}$ second or less [Table 1, column (a)] if it is assumed that the entire low-energy absorption band for each base is responsible for emission. If, however, the fluorescent oscillator is assumed to be the fluorescence excitation fitted to the low-energy absorption band [which may give an estimate of that portion of the total absorption actually responsible for fluorescence (*10*)], all lifetimes are significantly increased [Table 1, column (b)]. Singlet lifetimes at room temperature calculated directly from the quantum yield and the absorption spectrum must therefore be considered a lower limit. In addition, the slightly broader emission spectra reported for all the bases at 300°K relative to 77°K may mean a different overlap integral for singlet transfer. The role of singlet energy transfer at room temperature must remain an open question.

The O-O′ energies (Table 1) are in the order adenine ~ uracil > cytosine > thymine > guanine. Although different from the order for the nucleotides at 77°K (*16*), particularly in the position of guanine, our values are very similar to those determined (*17*) at an intermediate temperature of 195°K for adenine (3.56 μm^{-1}) and guanine (3.32 μm^{-1}) (*17*). Although room-temperature data on the nucleotides would be preferred for comparison, the results presented here may mean that the relative excited state energies of the bases are sufficiently altered by temperature to affect the direction of energy transfer.

In view of the significant differences in excited singlet properties between 77° and 300°K, extrapolation from low-temperature measurements to processes occurring in DNA under biological conditions may require careful reevaluation. Extension of the technique reported here to an investigation of the fluorescent properties of the nucleotides, excimer formation (*18*) in the dinucleotides, and the fluorescence behavior of DNA itself is now feasible.

MALCOLM DANIELS
WILLIAM HAUSWIRTH
Radiation Center and *Chemistry Department, Oregon State University, Corvallis 97331*

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Subunit Structure of Aldolase

Abstract. *A new crystal form of rabbit muscle aldolase shows that the molecule has 222 symmetry to at least 4-angstrom resolution, and hence that the gross conformation of the four subunits is the same. Comparison of the new form with a previously reported form establishes the number of molecules per unit cell,* n, *in the older form. For an independent check, the "crystal-volume and protein-content method" was developed to determine* n *without directly measuring the water content of the crystals.*

Aldolase from rabbit muscle is composed of four subunits, each having a molecular mass of about 40,000 daltons (*1*). Two crystal forms of this enzyme have been reported (*2, 3*): form I (Table 1) is a hexagonal form that is